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VOLUME 42

October-December, 1939 (inclusive)

NEW YORK

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Press of
THOMAS J. GRIFFITHS SONS, INC.,
Utica, N. Y.

ERRATUM

Article 10580, by Steele, 1939, **41**, 86: Reference 4 should read, 1938, **38**, 10; Reference 8 should read, Hamilton, W. F., Brewer, G., and Brotman, I., *Am. J. Physiol.*, 1934, **107**, 427.

Proceedings
of the
Society
for
Experimental Biology and Medicine

VOL. 42. OCTOBER, 1939. NO. 1.

SECTION MEETINGS

NEW YORK
New York Academy of Medicine October 18, 1939

10780

Alterations of the Cardiac Cycle and of Polygrams Produced by Artificial Fever.

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According to Neymann[1] "the heart or its functioning is not harmed by artificial fever produced by physical means." The observations of Bazett and coworkers[2, 3, 4] on the effects of prolonged exposure to heat clearly indicate that a warm, humid external environment gives rise to changes which cause cardiovascular strain and subjects the patient to 'incipient cardiovascular failure'. Their observations show that factors leading to the development of a severe reduction in the venous return of blood to the heart overbalance the compensatory mechanisms of the cardiovascular system and eventually bring about the development of cardiovascular inefficiency. Furthermore, their findings are in agreement with the view that dehydration and diminished blood volume explain quite satisfactorily the prostration and discomfort of patients exposed to high external temperatures of long duration (8 to 10 hours) but it seems unlikely that these factors are responsible for the development of the undesirable subjective and objective symptoms frequently encountered during the induction stage of artificial hyperpyrexia. Since this phase of the problem has not been exhaustively investigated we elected to make a study of it by means of pulse tracings.

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[1] Neymann, C. A., *Artificial Fever*, Charles C. Thomas, Baltimore, Md., 1938, 251.

[2] Bazett, H. C., *Med. Rec.*, 1938, **147**, 301.

[3] Bazett, H. C., *J. Am. Med. Assn.*, 1938, **111**, 1841.

[4] Bazett, H. C., Scott, J. C., Maxfield, M. E., and Blithe, M. D., *Am. J. Physiol.*, 1937, **119**, 93.

Studies on the cardiac cycle during fever therapy were made on human subjects. Optically recorded polygrams utilizing Frank capsules were combined with electrocardiograms. Central sphygmograms recorded from the subclavian or lower carotid artery were employed to determine the ejection phase of the cardiac cycle and phlebograms recorded from the subclavian or jugular vein were used to measure the various components of the filling phase. Artificial fever therapy was induced by means of an air-conditioned fever cabinet. The general procedure employed in our experiments was as follows: (a) control electrocardiograms of leads I, II and III were made; (b) control polygrams of the subclavian or jugular vein, subclavian artery, lower carotid, upper carotid, radial artery and apex beat were recorded simultaneously in groups of two on an electrocardiogram; (c) after finishing the control records, the subject was placed in the fever cabinet and the artificial fever started; (d) polygrams consisting of a central sphygmogram, a phlebogram and a lead II electrocardiogram were recorded at approximately each degree Fahrenheit during the elevation of body temperature up to and including 106°F.

Such observations were made on a series of 7 normal subjects and upon 4 patients who were receiving artificial fever therapy treatments. Our results may be briefly presented by citing some of the data obtained from the analysis of the polygrams thus recorded from a typical individual in the group. The pulse tracings of Subject No. 13 showed the following results: (1) The heart rate increased from 57 per minute to 140 per minute and the duration of the total cardiac cycle decreased from 1.05 seconds to 0.43 second. (2) The emptying time of the ventricles was progressively shortened from a normal duration of 0.34 to 0.18 second (53% of the normal emptying time). The reduced ejection phase was slightly more shortened than the maximal ejection phase. (3) The total filling time of the ventricles was rapidly reduced to 40% of the normal duration by the time the temperature was elevated to 100.5°F, and at this temperature level the filling time was less than the emptying time. Within a few minutes the filling time slightly increased in duration so that it again became greater than that of the emptying time, the body temperature in the meantime continuing to rise. (4) The constituent phases of ventricular filling were rapidly and more or less progressively diminished in duration as the body temperature was elevated, reaching their lowest respective levels at 102.5° to 103.5°F. The duration of the filling phases except for the phase of diastasis increased again as the temperature level was increased. (5) The phase of diastasis was often completely abolished at temperatures higher than 103.0°F. (6) Graphs constructed from the data showed that the duration of atrial systole and diastole practically parallel the ventricular isometric relaxation curve. These curves suggested the presence of similar functional changes in the behavior of the atrial musculature to those which presumably occurred in the ventricles during isometric relaxation. (7) The rapid filling and slow filling phases of the ventricles showed a marked reduction in the duration of the filling time at temperatures ranging between 102.5° and 103.5°F. Both the rapid and slow filling phase increased and remained somewhat prolonged though considerably less than the control filling time values. (8) The sphygmograms showed considerable alteration in size, shape and composition. They became more irregular in shape and were often definitely 'spiked'. The dicrotic notch became lower on the catacrotic limb, descending considerably below the base line. The dicrotic wave was frequently enlarged and prolonged. Additional anacrotic waves appeared and there were frequently several extra predicrotic and postdicrotic waves of frequencies ranging from 20 to 40 cycles per second. (9) The phlebograms showed considerable variation in size, shape and contour. The 'C' wave became accentuated, often converting an atrial type of phlebogram into an intact type. The vibratory waves of the second heart sound became exaggerated, the 'V' waves frequently became quite inconspicuous. Quite often there appeared to be no line of demarcation between the slow and rapid filling phases. The 'A' waves showed alterations in size. During the induction stage the 'A' wave frequently became relatively decreased in size but beyond 103 or 104°F, the 'A' waves again became relatively increased. These observations on the alterations of the 'A' wave of the phlebogram suggest that an increase in atrial activity may be a factor in cardiac compensation during the induction stage of artificial hyperpyrexia.

Conclusions. Analysis of the polygrams recorded during artificial fever suggest that the distressing symptoms one observes and the subjective discomfort experienced by patients during the induction of artificial hyperpyrexia are caused by an inadequate filling of the heart. The inadequate filling of the heart may be accounted for, in part at least, by an insufficient duration of the filling time. These observations further support the belief that the undesirable symptoms which occur during artificial fever therapy are caused by a temporary cardiac decompensation resulting from inadequate filling. Reduced cardiac filling probably results from (1) a reduced venous return resulting from uncompensated peripheral vasodilatation, (2) a shortening of the filling time, and (3) reduced blood volume.

Compensation may be accounted for not only by an increase in the efficiency of the emptying of the ventricles but also by a more adequate filling brought about by an increase in the venous return, an increase in the duration of the filling time and possibly by increased atrial activity.

10781

A Histological Problem Concerning the Conditions at the Nerve Endings in Skeletal Muscle.

H. A. BLAIR AND SIBYL STREET. (Introduced by W. O. Fenn.)

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Until the recent appearance of the chemical transmission hypothesis[1] it was generally assumed that the excitation of skeletal muscle was effected electrically by the action current of the nerve ending. At present neither the chemical nor the electrical hypothesis can be considered to be well established. It seems likely from the nature of the problem that much of the decisive evidence must come from a demonstration that the structures and arrangements at the neuromuscular junction are more suited to the one type of transmission than to the other. In any case, the point of view must be consistent with respect to the structure of the junction and its function. It is our purpose to discuss a serious inconsistency which exists at present in regard to the electrical polarization of the membrane of nerve ending.

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[1] Dale, H. H., Feldberg, W., and Vogt, M., *J. Physiol.*, 1936, **86**, 353.

The polarization of the membranes* of both nerve and muscle is believed to be a consequence of semi-permeabilities. The membranes are impermeable to anions while permeable to certain cations, notably to K which is the principal cation within the cell but which exists in relatively low concentration in the extracellular fluid. The K ions are retained within the cell for the most part in association with the impermeable negative ions thus providing a concentration difference from inside to outside which accounts satisfactorily for the observed membrane potential.[2, 3] It is believed that in the case of muscle the polarized membrane is associated with the sarcolemma or a plasma membrane immediately underlying it. The nerve according to the most generally accepted histological description penetrates the muscle membrane after losing its sheaths and lies embedded in the specialized sarcoplasm of the end plate. Thus Fig. A probably is in best accord with current histological and physiological opinion. Here the nerve membrane is represented as being polarized after it penetrates the polarized muscle membrane. This representation is, however, quite impossible according to the K hypothesis for if both sarcoplasm and neuroplasm are of similar high K content there can be no appreciable difference of K concentration across the membrane of the nerve ending and consequently this membrane cannot be polarized. But if the nerve ending is not polarized after it enters the sarcolemma it cannot, according to current views, convey a nerve impulse electrically, neither can it act as a secretory organ in accord with the chemical hypothesis, because organs known to secrete in response to stimulation do so on account of or accompanied by membrane depolarization.

This difficulty can, conceivably, be avoided in a number of ways. According to Fig, B, for example, it may be supposed that the polarizable membranes of the nerve and muscle are actually in contact. But in the contiguous regions the K gradients across the membranes and consequently the polarizations will be suppressed. However, the polarized regions of the nerve and the muscle will be essentially continuous over the boundary of the contiguous region permitting easy electrical conduction. It might be expected, however, that this arrangement would permit as much diffusion into the muscle of a

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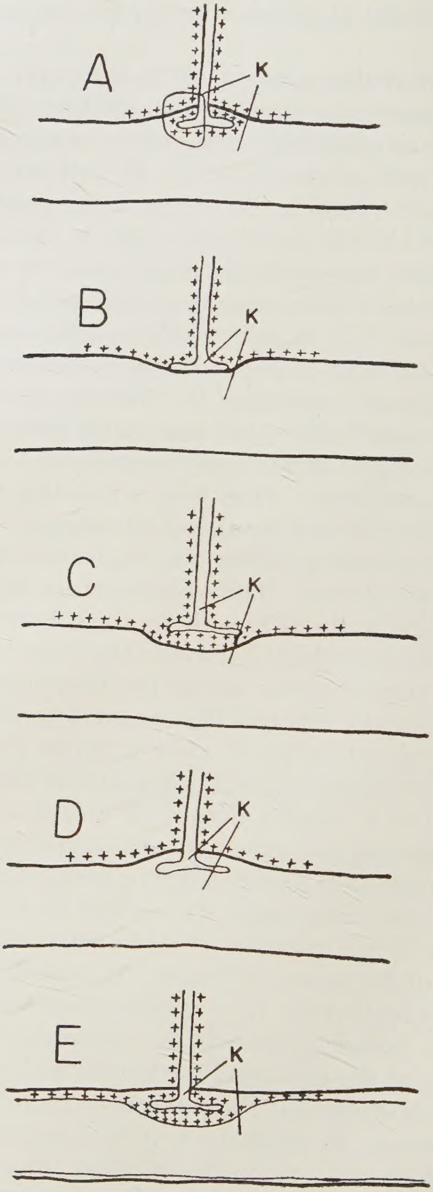
* Although there is no exact indication from electrical measurements of the location of the polarization it must from physico-chemical considerations, almost certainly be across the cell membrane and not across connective tissue sheaths.

[2] Fenn, W. O., Cobb, D. M., Hegnauer, A. H., and Marsh, B. S., *Am. J. Physiol.*, 1934, **110**, 74.

[3] Fenn, W. O., *Physiol. Rev.*, 1936, **16**, 450.



FIGS.

In Fig. A is represented schematically the current concept of the positions of the polarized membranes of the muscle and nerve as indicated by the plus signs. The other figures depict alternative arrangements. In Fig. E the inner lines represent the boundaries of the myofibrils. In all cases the lines from the letter K point to regions rich in potassium. The figures are discussed in the text.

chemical formed in the nerve at one time as at another so it would not be well adapted to chemical transmission.

Another possibility is depicted in Fig. C, in which it is supposed that the polarized membrane of the muscle fiber is not penetrated by the nerve, but folds in around the ending, leaving access for free circulation of extra-cellular low K fluid. The space between muscle and nerve substance would probably need to be of the same order as that separating nerve fibres in a nerve bundle. This arrangement would permit the nerve endings to be polarized throughout and would probably be more favorable for chemical than electrical transmission for reasons which need not be discussed here.

Still another possibility is indicated in Fig. D. In this case which is essentially a correction of the current concept in Fig. A, to allow for high K around the ending it is assumed that the membrane of the nerve ending is not polarized after entering the sarcoplasm and that the polarizable membranes of the nerve and the muscle come into contact at the point of entry permitting a continuous polarization over the junction. This arrangement which is in best accord with histological opinion, presumably would be admirably suited to electrical, but not to chemical transmission for the reasons given in connection with Fig. B. In this case, the nerve ending could be assigned only trophic functions since it would not be necessary for transmission.

A fifth possibility is represented in Fig. E. Here it is assumed that the high K region of the muscle is within the myofibrils and not in the sarcoplasm. In this case the polarized membrane of the muscle would be around the fibrils only and the nerve endings being surrounded by low K would be polarized and capable, therefore, of conveying impulses to their termini and effecting electrical or more probably, chemical transmission. The existence of this possibility is supported by the evidence for a periterminal network connecting the myofibrils and the endplate but opposed by studies on embryos[4, 5] which suggest that nervous stimulation of a muscle fiber is possible as soon as a motor nerve makes contact with its outer membrane. Also, as mentioned above, high K is thought to occur in the sarcoplasm on the basis of chemical work.

It cannot be denied, of course, that there is a possibility that the membrane polarization of the nerve ending is maintained by other means in an arrangement like Fig. A, even within a sarcoplasm of high K content. This is perhaps rather unlikely, however, and in addition this arrangement would give rise to a continuous current flow if the membrane potentials of the nerve and the muscle were

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[4] East, E. W., *Anat. Rec.*, 1931, **50**, 201.

[5] Tello, J. F., *Z. f. Anat. u. Entwicklunges*, 1922, **64**, 348.

similar in magnitude. For a line of possible current flow, as indicated by the loop on the left in Fig. A, starting within the nerve ending and crossing first the sarcoplasm, then the muscle membrane and then the nerve membrane again outside the muscle would encounter 2 polarizations in one direction and only one in the other. Consequently, the potential would not be balanced and current would flow continuously.

A discontinuity between the polarized membrane of the muscle and that of the nerve in the Fig. D arrangement would also allow local currents to flow from each membrane through the discontinuity. The smaller the discontinuity, however, the less the current and there would seem to be no reason why the discontinuity and, therefore, the current might not be thought to be vanishingly small even if the way were not clear for assuming a strict continuity of the nerve and muscle membranes.

The arrangements in Fig. B and D would probably permit electrical conduction both from nerve to muscle and from muscle to nerve. This would not raise any physiological difficulty however, because, since the nerve is refractory longer than the muscle, it is improbable that an impulse would be reflected, as it were, to return up the nerve again.

It appears from the literature that while the structure of most of the components of the motor nerve endings have been studied intensively, the relations between the physiologically important membranes have received little or no attention. This problem is presented, therefore, in the hope that some histological or embryological inquiry with the physiological questions in mind may yield more conclusive evidence than is afforded by the existing physiological data as to whether the neuro-muscular transmission is more probably chemical or electrical. Also any light which may be thrown on the relations of the polarized membranes at these or other synapses will be, for reasons similar to those discussed here, of the greatest assistance in developing ideas as to the nature of synaptic transmission in general.

10782 P

Standardization of the Anti-viral Content of Immune Globulin from Placental Extract.

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It is now accepted that preparations of globulin from human placentas can modify or prevent measles in humans.[1] In addition to its content of anti-measles antibody indicated clinically, the placental extract has been shown to contain diphtheria and scarlet fever antitoxins and antipoliomyelitis antibodies.[2] At the present time the diphtheria antitoxin content is widely employed as a means of determining the potency of different preparations; and, despite the fact that the antitoxic and antiviral antibodies are distributed differently in the placental extract, with the former entirely in the pseudoglobulin and the latter distributed in several fractions,[3] it has been stated that the determination of the diphtheria antitoxin titer is satisfactory as a basis for determining the anti-measles potency of different preparations.

Ideally, each preparation should be standardized for its anti-measles antibody content. Since, however, the lack of a susceptible animal other than the monkey renders such standardization at present impossible, it would seem advisable to titrate the different preparations for some other antiviral antibody. Poliomyelitis virus neutralization tests have been successfully carried out[3] but here again no routine standardization is possible. Antibodies for other viruses are to be found in human sera, however, and those against swine influenza are probably as widespread as those against measles. These influenza antibodies are readily titratable in the albino Swiss mouse and different preparations of placental extract can be tested for this antiviral antibody. Such tests are reported below.

Lungs of mice, sacrificed while moribund from swine influenza infection* after intranasal inoculation and showing extensive lung involvement, were used after being preserved in 50% glycerine from 1-5 days. Such lungs were ground in sufficient tyrodes to give a 1/50 dilution of the wet weight of the lung. Half cc amounts of the 1/50 suspension of virus were mixed with ½ cc amounts of the placental extract dilution to be tested and the mixture allowed to stand for 30 minutes at 37°C. As a control a saline solution of preservative at the same strength as that of the maximum present in any one of the placental extract dilutions was mixed and incubated with the virus in the same way. 0.04 cc amounts of these incubated mixtures were given intranasally to anesthetized Swiss mice, using a minimum of 4 mice for each dilution tested. The mice were observed for 8 days and then sacrificed and examined for lung lesions. Table I shows typical results. Here dilutions of these 2 placental extracts showed some protection at 1/80 and good protection at 1/20. One specimen of placental extract P^1 has been chosen as a standard to which all others may be referred. After several titrations of its potency it has been given a provisional titer of 100 units per cc.

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[1] Council on Pharmacy and Chemistry, *J. A. M. A.*, 1938, **111**, 1764.

[2] McKhann, C. F., and Chu, F. T., *J. Inf. Dis.*, 1933, **52**, 268.

[3] McKhann, C. F., Green, A. A., and Coady, H., *J. Pediat.*, 1935, **6**, 603.

* The swine influenza virus was received from Dr. R. E. Shope to whom for this and many helpful suggestions I wish to express my thanks.

TABLE I.

Placental preparation	1/5				1/20				1/80			
243-254	0*	0	0	0	0	0	0	0	+++†	++	++	++
257-268	0	0	0	0	+	0	0	0	+++	++	++	+
Control					4‡	5	5	6				

‡ Mouse died with characteristic lung lesions on the 4th day after inoculation.

†Mouse killed on the 8th day and showed approximately ¾ of the lungs consolidated (++ = approximately ½ of the lungs consolidated).

*Mouse killed on the 8th day and showed normal lungs.

This method of titration has been used to follow the distribution of antiviral antibodies during the purification and concentration of the placental extract. Table II shows the results of the testing of various fractions. It will be seen that fractions C, D, and E contain no neutralizing antibodies; A, B, and F contain varying amounts. The technic used was that given above. All survivors were sacrificed on the 8th day and examined for lung lesions.

TABLE II.

A	+++	0	0	0
B	+++	+++	+++	++
C	4	5	5	6
D	5	5	5	6
E	4	5	5	7
F	+++	+++	++	±
Control	4	5	5	7

It was important to show that the neutralization being obtained was due to specific antiviral antibodies. There was a possibility of a non-specific destruction of the virus by hypothetical toxic substances in the placental extract. This supposition was rendered improbable by the fact that, in following up the fractionation of the placental extract, no activity was found in those fractions which have been shown clinically to have no anti-measles activity. As a first control of this theoretical objection it has been shown that the same placental extract preparations which protect when used in the technic given above also protect when they are given to the mouse 15 minutes before the virus suspension. Thus the mixing and standing of placental extract and virus for 30 minutes at 37°C is eliminated. Furthermore, neutralization tests were carried out with a virus, Eastern Equine Encephalomyelitis, antibodies for which are not to be expected generally distributed in the sera and placentas of normal adult females. The strain used was killing mice regularly through 10^{-8} and over 50% at 10^{-9}. Fresh mouse brain was used from a mouse moribund with the infection. Dilutions of the brain were made in broth through 10^{-11}. For testing the placental extract samples the dilution 10^{-8} was used. Half cc amounts of this were mixed with ½ cc amounts of the different fractions and of the merthiolate control, and the mixture placed at 37°C. for 30 minutes. Then 0.04 cc of each mixture was inoculated intracerebrally in each of 4 normal Swiss mice. Control titrations of the virus alone were made at 10^{-7} and through 10^{-11}. Table III shows the results. No neutralization is to be observed.

TABLE III.

A	42,* 68, 90, 90	Virus Titration	
B	43, 48, 66, 66	10^{-7}	43, 43
C	43, 43, 48, 48	10^{-8}	48, 66
D	48, 48, 66, 72	10^{-9}	66, 66, S†
E	66, 66, 72, 90	10^{-10}	S, S, S
F	48, 66, 66, 66	10^{-11}	S, S, S
Control	48, 66, 66, 66		

*Died in 42 hours.
†S = Survived.

Thus a method of titrating the antiviral neutralizing antibodies of placental extract for the swine influenza virus has been demonstrated. This is believed to be a specific antiviral action and not due to non-specific toxic fractions in the placental extract. The suggestion is made that standardization of the antiviral antibodies of placental extracts by this method may prove a better indication of the anti-measles content of such extracts than does the present method of titrating the diphtheria antitoxin content. The adoption of a provisional standard extract and provisional unit will allow further investigation to be carried out on a quantitative basis.

10783

Protective Effect of Sulfapyridine, Sodium Sulfapyridine, and Sulfanilamide in Pneumococcus Infection Types I, II, and III.

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The successful clinical use of sulfapyridine in the treatment of pneumonia was stimulated by Whitby's experiments on mice. Whitby[1] was able to prolong the life of mice inoculated with various types of pneumococci for 7 days, while with sulfanilamide the average survival was 1.3 to 3.3 days.

Fleming[2] found that sulfapyridine *in vitro* retards the growth in human blood of pneumococci in concentrations which it is reasonably supposed can be obtained therapeutically. MacLean, Rogers, and Fleming[3] found that the sensitivity to sulfapyridine of different strains belonging to the same type of pneumococcus varied considerably. Types I, II, and III were most resistant.

In the experiments of Cooper, Gross and Lewis[4] sulfanilamide-treated mice showed a survival of 25%; with sulfapyridine 45% survived. Our results are more in accord with those of Long, Bliss, and Feinstone,[5] who reported that with a highly virulent strain of pneumococcus type I they obtained 100% mortality with sulfanilamide, and 88% mortality when sulfapyridine was used.

Chemistry. Sulfapyridine is closely related to sulfanilamide, a hydrogen of the amido group being replaced by the pyridyl group. Its chemical properties were described by us in a previous paper.[6] The sodium salt of sulfapyridine was first described by Marshall, Bratton, and Litchfield.[7] This product was prepared independently by us. It is a colorless, crystalline substance, easily soluble in water, insoluble in organic solvents.

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[1] Whitby, L. E. H., *Lancet*, 1938, **1**, 1210.

[2] Fleming, A., *Lancet*, 1938, **2**, 74.

[3] MacLean, I. H., Rogers, K. B., and Fleming, A., *Lancet*, 1939, **1**, 562.

[4] Cooper, F. B., Gross, P., and Lewis, M., Proc. Soc. Exp. Biol. and Med., 1939, **40**, 37.

[5] Long, P. H., Bliss, E. A., and Feinstone, W. H., *Pa. Med. J.*, 1939, **42**, 483.

[6] Raiziss, G. W., Severac, M., Moetsch, J. C., and Clemence, L. W., Proc. Soc. Exp. Biol. and Med., 1939, **40**, 434.

[7] Marshall, E. K., Jr., Bratton, A. C., and Litchfield, J. T., Jr., *Science*, 1938, **88**, 597.

TABLE I.
Toxicity of Sulfanilamide, Sulfapyridine, and Their Derivatives in Rabbits *Per Os* and Intravenously.

Drug	Dose per kilo (g)	No. of animals	Survivals		Deaths	
			No.	%	No.	%
Sulfapyridine*	1.5	7	7	100		
	2.0	6	4	66⅔	2	33⅓
Sulfanilamide*	1.5	18	17	94	1	6
	2.0	16	8	50	8	50
Sodium sulfapyridine†	0.5	3	3	100		
	0.75	3	1	33⅓	2	66⅔
	1.0	2			2	100
Sodium sulfanilamide†	0.2	1	1	100		
	0.75	1	1	100		
	1.0	2	2	100		
	1.25	2			2	100
	1.5	1			1	100

**Per os.* †Intravenously.

Toxicity. 100% of rabbits tolerated 1.5 g of sulfapyridine per kilo given orally, while 66% survived a dose of 2 g per kilo; 94% tolerated 1.5 g and 50% tolerated 2 g of sulfanilamide per kilo. Sulfapyridine is, therefore, slightly less toxic than sulfanilamide. Intravenously, the sodium salt of sulfapyridine was tolerated in a dose of 0.5 g per kilo, compared to 1 g per kilo for sodium sulfanilamide. Sulfanilamide, therefore, is the less toxic of the two drugs intravenously. (Table I.)

Therapeutic effectiveness in pneumococcus types I, II, and III. Two strains of each of the 3 types of pneumococcus were used. Strain 37 of type I pneumococcus, 1599, type II, and 1460, type III, were obtained from Sharp and Dohme.* Strain M30, type I, M48, type II, and M50, type III pneumococcus were supplied through the courtesy of Dr. Perrin Long, of Johns Hopkins Hospital, who considered them very virulent. We found the Sharp and Dohme strains equally as virulent. These strains usually killed mice in a dilution of 10^{-7} (1:10,000,000) to 10^{-8} (1:100,000,000). Mice were infected intraperitoneally with 200 minimum lethal doses of the culture. Many individual experiments were made, each compound being tested on 5 or 10 mice. In Table II are presented summaries of the results obtained in these individual tests. Pneumococci types I, II, or III were subjected to 3 different systems of testing. In the first system only 5 treatments of the drug were given, in the second 10, and in the last a maximum of 18 (Long's method). Further modifi-

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* Philadelphia, Pa.

TABLE II.

Therapeutic Effect in Types I, II, and III Pneumococcic Infection in Mice.
Each treatment consisted of 0.010 g of the drug by mouth.

Type	Strain	Drug	No. of mice	Percentage of survivals in days									
				1	2	3	4	5	6	7	14	21	28
I	37†	Sulfapyridine	20	100	90	45	30	15	5	5	5	5	5
		Sulfanilamide	10	100	70	20	0						
		Sodium sulfapyridine	5	100	100	100	60	0					
	M36†	Sulfapyridine	25	96	80	12	4	4	0				
		Sulfanilamide	15	100	47	0							
		Sodium sulfapyridine	15	100	93	47	33	27	27	27	20	20	13
	M36‡	Sulfapyridine	10	100	100	50	30	30	20	20	10	10	10
		Sulfanilamide	10	30	0								
		Sodium sulfapyridine	10	100	90	80	80	80	60	60	40	30	30
II	1599*	Sulfapyridine	30	100	70	30	23	13	3	0			
		Sulfanilamide	15	80	0								
	1599†	Sulfapyridine	70	100	100	61	21	14	8	7	7	7	7
		Sulfanilamide	45	89	25	0							
		Sodium sulfapyridine	10	100	90	80	20	10	10	0			
	M48‡	Sulfapyridine	20	100	95	75	30	22	17	8	2.5	0	
		Sulfanalimide	20	90	68	13	5	0					
		Sodium sulfapyridine	15	100	100	85	60	53	47	3	0		
III	1460†	Sulfapyridine	30	84	70	37	23	23	20	20	20	20	20
		Sulfanilamide	20	70	30	10	5	5	5	5	5	5	5
		Sodium sulfapyridine	15	100	93	47	33	20	20	20	20	20	20
	M50†	Sulfapyridine	5	100	80	20	0						
		Sulfanilamide	5	80	0								
		Sodium sulfapyridine	5	100	80	40	0						
	M50‡	Sulfapyridine	10	100	100	80	60	40	40	40	10	0	
		Sulfanilamide	10	90	30	10	0						
		Sodium sulfapyridine	10	100	100	100	60	40	40	30	20	20	20

*Treated 1½ hr after infection, and once daily until total of 5 treatments. †Treated 1½ and 6 hr after infection first day, twice on second day, and once daily until treatments total 10. ‡Treated immediately, 6 and 12 hr after infection, three times daily for 4 days, twice daily for one day, and once on the last day.

cations concerned the time of treatment, that is, immediately following infection or 1½ hours after, also the number of daily treatments, namely, once, twice or 3 times a day. The dose in every treatment has been invariably 10 mg.

Pneumococcus type I. When strain 37 was used sulfapyridine in the first 5 days was effective in prolonging life, but on the sixth day only 5% of mice survived. This shows then that sulfapyridine prolongs the life of mice, but cannot prevent death. Sulfanilamide kept 20% of infected mice alive for 3 days; on the 4th day all were dead. Sodium sulfapyridine showed a good therapeutic effect until the 4th day, 60% of mice surviving, but all died on the fifth day.

All mice treated with sulfanilamide were dead on the third day when strain M36 was used. Of the sulfapyridine-treated mice, 80% on the second day, but only 12% on the third, survived; by the sixth day all animals were dead. With sodium sulfapyridine, 93% of the mice lived for 2 days, 47% for 3 days, and 13% for 28 days.

Substantially better results were obtained for sulfapyridine when the same strain, M36, was used with more intensive treatment, starting immediately after infection; 10% of mice remained alive at the end of the period of observation. Sulfanilamide-treated mice were dead on the second day. Comparatively good results were obtained with sodium sulfapyridine, 60% of mice surviving 7 days, and 30% living at the end of 28 days.

Pneumococcus type II. Of the 30 mice infected with pneumococcus type II, strain 1599, and treated 1½ hours after infection with sulfapyridine, only 23% survived after the fourth day. After 6 days only 3% were alive. No animals treated with sulfanilamide were living at the end of 2 days.

In mice infected with the same strain (1599), but given 10 treatments instead of 5, the effect of sulfapyridine was a little better after 6 days, 8% of animals surviving instead of 3%; 7% survived 28 days. While there is a slight improvement due to the longer treatment, the difference in the therapeutic result is comparatively small. With sulfanilamide, all animals were dead on the third day. Sodium sulfapyridine had about the same effect as sulfapyridine, except that after 6 days of observation all animals were dead.

When mice were infected with strain M48, and treated more intensively than in the previous series of experiments, starting immediately after infection, sulfapyridine showed 17% survival after 6 days. However, after the 14th day, most of the animals began to die, so that on the 21st day none were alive. The intensive treatment did not materially change the therapeutic effect. Sulfanilamide caused a prolongation of life, although it was small; all animals died by the 5th day. Sodium sulfapyridine kept more animals alive for six days, but on the 7th day only 3% survived.

Pneumococcus type III. Of mice infected with strain 1460 and treated 1½ hours after infection with sulfapyridine, 20% survived on the sixth day; the same number survived on the 28th day. Sulfanilamide also showed better results, 5% of mice surviving 28 days. Sodium sulfapyridine showed the same survival rate as sulfapyridine.

When strain M50 was used, results were inferior to those obtained with strain 1460. All mice were dead on the 4th day with sulfapyridine, on the 2nd day with sulfanilamide, and on the 4th day with sodium sulfapyridine.

In another series of experiments animals infected with strain M50 were treated intensively by Long's method. Treatment was given immediately after infection, 6 and 12 hours later the 1st day, 3 times daily for the next 4 days, twice for one day, and once on the last day. Of mice treated with sulfapyridine, 40% were living on the sixth day, but after 14 days almost all were dead. All sulfanilamide-treated mice were dead on the 4th day. Sodium sulfapyridine showed better results than sulfapyridine, 20% of mice surviving on the 28th day.

Blood cultures. Blood was taken from the tail of mice infected with pneumococcus type I and subcultured on blood agar plates. In

TABLE III.
Presence of Pneumococcus Type I in Blood Cultures* of Mice Treated with Sulfapyridine and Allied Products.

Drug	Mouse Number	Hours after treatment			After 5 days	Heart culture
		24	48	72		
Sulfanilamide	4	pos.	died			positive
	5	”	”			”
Sulfapyridine	1	”	pos.	died		”
	2	”	”	”		
	3	”	”	pos.	pos.	
	4	”	”	died		”
	5	”	”	pos.	”	
Sodium sulfapyridine	1	neg.	neg.	died		
	2	”	”	pos.	neg.	
	3	pos.	”	neg.	”	
	4	”	pos.	”	”	
	5	neg.	neg.	”	”	
Controls	1	pos.	pos.	died		
	2	”	”	”		

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*Routinely the blood for cultures was taken from the tail of the animal; in case of death the blood from the heart was cultured.

case of death, the blood from the heart was cultured. In mice treated with sulfapyridine, the blood was positive throughout 5 days of examination. Pneumococci were present in the blood of mice after 24 hours' treatment with sulfanilamide; after 48 hours mice were dead, and the heart culture proved positive for pneumococci. Mice receiving sodium sulfapyridine, however, showed a number of negative results for pneumococci during 5 days of observation. (Table III.)

Conclusions. 1. Sulfapyridine administered orally to rabbits is slightly less toxic than sulfanilamide. 2. Sodium sulfapyridine given intravenously is considerably more toxic than sodium sulfanilamide. 3. While the protective effect of sulfapyridine in pneumococcal bacteremia types I, II, and III is small, 20 to 30% of mice surviving on the 4th day and less than 10% on the 7th day, it is somewhat higher than that afforded by sulfanilamide, which results in an average survival of 2 to 3 days. Sulfapyridine prolongs life of infected mice only for an additional period of 2 to 4 days. The protective effect of sodium sulfapyridine is of the same order as that of sulfapyridine.

10784

Monophasic Action Currents from the Uninjured Turtle Ventricle.

Richard Ashman and Norman C. Woody.

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Monophasic action currents obtained by derivation from 2 uninjured points on heart muscle have often been mentioned in the literature. Samojloff,[1] for example, produced a block by transverse cutting or compression of the frog heart and recorded monophasic action currents by derivation from uninjured base and apex. In this experiment, however, the apical muscle constitutes an extension of the lead to the injured surface. DeBoer[2] has recently stressed his observation that, employing base-apex leads in the frog, the weak beat in alternation may be associated with monophasic action currents. Although no injury was produced in these experiments by mechanical means, the blocking of the impulse must have been due to the presence of muscle in an abnormal physiological state. Gilson[3] has recorded monophasic action currents following injury, but at a time when the demarcation current had shrunk practically to zero.

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[1] Samojloff, A., *Arch. f. d. exp. Physiol.*, 1914, **155**, 471.

[2] De Boer, S., *Cardiologia*, 1938, **2**, 292.

In our observations, which are incidental to a study of the action currents of the turtle heart, monophasic action currents were produced as follows. The whole heart was excised. A thread was tied to the frenum, care being taken not to injure the apex. Threads were attached to the great arteries, which were cut near the ventricle, and to the apex of each auricular appendage. Each of the 3 threads, excluding that at the frenum, was tied to a very thin strip of rubber elastic by which the heart was suspended from a rod. The heart was partly lowered into a small beaker. The apical thread was then passed under a hook attached to the floor of the beaker by cardboard and paraffin, and fixed outside the beaker. Thus, on contraction, the base of the heart was drawn down, and the apex was relatively immobile. Fine wires from an inductorium were inserted into an auricle. Ringer's solution at room temperature was then poured into the beaker until the apex was slightly immersed. Galvanometer contacts consisted of wicks of absorbent cotton, connected to Zn-$ZnSO_4$ "boot" electrodes, one dipping into the Ringer's, the other resting on the ventricular base. Electrograms were then recorded, of both sinus and premature beats, the latter being induced by auricular stimulation. The diastolic base line was also recorded. No compensating current was used. Each curve was standardized in 3 millivolt steps. The Ringer's solution was then siphoned off and replaced by Ringer's, cooled to nearly 0°C. In one experiment, with typical results, the Ringer's solution was not cooled to below 5°C.

In the 5 hearts used with this procedure, monophasic action currents were recorded. These were wholly unassociated with any consistent change in the diastolic line of zero potential difference. The observed changes, indicating either slight positivity or slight negativity of the resting apex, were almost negligible in comparison with the recorded action potentials. The QRS complex, in the uncooled heart, consisted of an upright "R" wave, sometimes followed by a small "S" wave. Except in one experiment (F in the figure), after slight, inadvertent injury of the extreme apex, the RS-T segment was within 2 millivolts of the base line. The T wave was sometimes inverted, sometimes upright.

On cooling the apex, the T, if inverted before, became much deeper and wider. If originally upright, it became inverted and deep. In one experiment, slightly impure monophasic action currents, in a direction indicating apical positivity, were obtained in association with spontaneous premature beats. Later, from this same heart, slightly impure monophasic action currents appeared with every

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[3] Gilson, A. S., Jr., personal communication.

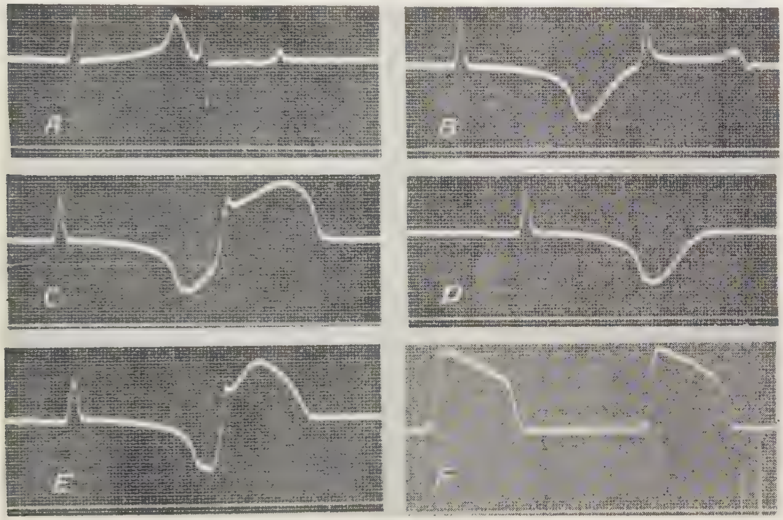

FIG. 1.

Records A to E, inclusive, for experiment of May 13, 1939. F, from experiment of May 6, 1939.

A, control. Apex in Ringer's solution at room temperature. A very small, upright P wave is seen, followed after 0.45 sec. by the initial ventricular complex. The RS-T segment lies practically on the isoelectric level. The artefacts on the early T upstroke are make-break shocks applied to an auricle to induce the premature response of the ventricle which begins late on the T downstroke.

B to E, inclusive, taken while apex is cold, and soon after A. In B the line of resting potential difference has descended just 1 mm., corresponding to 1.875 millivolts relative negativity of the apex. Later (D and E) this returns to the original level. The "S" wave of the initial deflections has disappeared. The T wave, previously upright, is now a large, downward deflection. The signal of auricular stimulation appears on the upstroke of this T in B. The premature response of the ventricle is essentially diphasic, because the wave of excitation has activated most of the apical muscle. In C, the premature response begins earlier. The electrogram is nearly, but not purely, monophasic. This electrogram is longer than the diphasic curve in A, partly because the base of the ventricle has rested longer; partly because the base may be slightly cooled. In D, a few beats after C was recorded, a diphasic response is recorded for comparison with E. Time, between D and E strips, 1.8 sec. In E, the premature response of the ventricle begins at almost the trough of the T wave. This curve is almost, but not quite, purely monophasic. The shoulder after the slight spike, is chiefly due to the superposition of the curve on the end of the T wave of the previous beat. The amplitude of this curve corresponds to 37.5 mvt.

F shows practically pure monophasic curves, from a more rapidly beating heart, which developed after the apex was cooled. The resting level shows 2 mvt. relative negativity of the apex. The diphasic form was restored when the apex was again brought to room temperature.

Standardizations: A to E, 1 cm = 18.75 mvt. F, 1 cm. = 20 mvt.

Time in 0.2 sec and 0.04 sec intervals.

The white line at the bottom of the curve gives a fixed reference level. No compensating current was employed in either experiment.

second sinus beat, a two-to-one block presumably having developed between base and apex because of the prolonged refractory period of the latter. In another experiment, practically pure monophasic action currents were associated with every beat for a time. In the other experiments, the beats of sinus origin were typically diphasic, but premature beats, induced between the trough and end of the T wave, were monophasic. (Fig. 1.) In no instance was any pure or nearly pure monophasic curve followed by a T wave. The duration of the monophasic curves was much less than the duration of the diphasic curves from the cooled heart, but they were slightly longer than the prematurely induced diphasic curves recorded before cooling. This prolongation may quite safely be attributed to the fact that there was always some cooling of the whole heart. Except for a greater or less rounding of the crest of the upstroke, the monophasic form was exactly similar to that induced by injury, and of the same order of magnitude. Typical diphasic curves were restored when the apex was returned to room temperature.

Although we were unable to prove that the occurrence of these monophasic action currents was associated with failure of response of the cooled surface, this interpretation seems to be almost certain, in view of the known blocking effect of cooling and from other considerations. It would be a simple matter to repeat the experiment with muscle strips, cooled at one end, but the necessary injury involved in cutting a strip complicates the interpretation.

These experiments demonstrate, we believe, that the only essential condition for the recording of monophasic action currents is the presence of a non-responding membrane (or surface). We have evidence to show that such a membrane, feebly polarized, appears, or is formed, at an injured surface. Its presence sufficiently accounts for the positive phase of the "injury action potential."[4] With cooling, there is no current of injury. The whole monophasic deflection is positive. Failure of response may be induced by injury, by cooling, by anesthetics, by KCl. The effects are essentially the same in all cases. This fact is difficult to reconcile with the conception that the monophasic action current is a specific effect of an injury, or of local depolarization of the resting muscle.

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[4] Eyster, J. A. E., Meek, Walter J., Goldberg, H., and Gilson, W. E., *Am. J. Physiol.*, 1938, **124**, 717.

10785 P

Effect of Carbon Tetrachloride on the Serum Amylase of Dogs.

J. O. Hanson. (Introduced by Warren H. Cole.)

From the Department of Surgery, University of Illinois College of Medicine, Chicago.

Following Somogyi's[1] observations of low serum amylase levels in individuals with liver damage, similar changes were observed at the Research and Educational Hospitals in patients coming to autopsy with varying degrees of hepatic insufficiency. This has stimulated an attempt to produce various types of liver injury experimentally in dogs to determine whether or not the changes in the serum amylase parallel changes in the liver closely enough to justify its use as an index of liver function.

The normal values for serum amylase in dogs are remarkably higher than those seen in humans. The normal readings obtained in these experiments ranged between 1900 and 3600, expressed as milligrams of sugar per 100 cc of serum. Each dog apparently has his own level of normal variations.

In the determination of the serum amylase, 5 cc of 1.5% starch paste was added to 2 cc of 1% sodium chloride solution and 1 cc of 1 to 10 diluted fresh blood serum and incubated at 39°C for 30 minutes. At the end of this time, the diastatic activity is ended by the addition of 1 cc of 5% copper sulfate solution. The proteins are precipitated by the Somogyi copper method. The sugar of the filtrate is determined by the quantitative method of Benedict. The control on the starch alone and the normal blood sugar are deducted, and the diastatic activity is expressed as the amount of reducing sugar in terms of milligrams of dextrose produced by 100 cc of blood serum under the conditions mentioned.

After the normal range for each dog had been determined, they were given varying amounts of carbon tetrachloride by gavage. The same general house diet has been used throughout.

In the first dog, it was noted that doses of 2 to 4 cc could produce a considerable drop in the serum amylase, but that in a day or 2 the level would rise again. After the administration of 38 cc of carbon tetrachloride, the serum amylase had dropped from an average normal of 2400 to a low point of 650. After one week of rest, the serum amylase level had returned to normal. (See Fig. 1.)

The average normal serum amylase in the second animal was 3000.

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[1] Somogyi, M., Proc. Soc. Exp. Biol. and Med., 1934, **32**, 538.

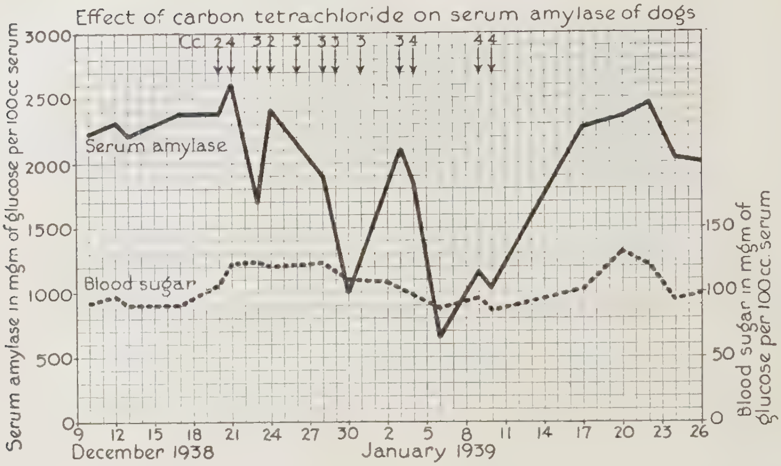

FIG. 1.

The heavy line represents the serum amylase curve, the lighter line the blood sugar curve. After the normal range had been established, carbon tetrachloride was administered in small doses of 2-4 cc every 2 to 3 days.

While the general trend was downward, wide fluctuations occurred. After one week of rest the amylase level was back to normal. These fluctuations and the return to normal may represent the reparative ability of the liver.

The blood sugar was unaffected by the carbon tetrachloride.

Larger doses were used in this dog, a total of 80 cc of carbon tetrachloride being given in 10 cc doses every 3 days. At present, the amylase level in this dog is down to 1200.

In the third dog, still larger doses—20 cc every 3 days—were administered with less striking results. Here too, however, the drop has been considerable, going from an average normal of 1900 to a low at present of 900.

The animals have acquired the ability to regurgitate the larger doses, and now the carbon tetrachloride is being given subcutaneously with better results, though with rather severe local reactions.

The effect of carbon tetrachloride on the livers of dogs has been best demonstrated by Bollman and Mann.[2] As yet, there is no proof that the drop in serum amylase is due to the liver destruction, but if similar results can be obtained by producing liver damage through other means, the evidence should be indicative of a close relationship between the serum amylase and the liver. There was no effect on the blood sugar levels.

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[2] Bollman, J. L., and Mann, F. C., *Ann. Int. Med.*, 1931, **5**, 699.

10786

Microtechnical Demonstration of Phosphatase in Tissue Sections.*†

G. Gomori. (Introduced by G. F. Dick.)

From the Department of Medicine, University of Chicago.

In preliminary experiments the phosphatase activity of aqueous extracts of dog kidney cortex made (a) of fresh ground tissue, (b) of ground tissue dehydrated with several changes of 95% and absolute alcohol, (c) of dehydrated ground tissue exposed to 56°C for one hour was determined by the Berenblum-Chain[1] modification of Kay's[2] method. These experiments have shown that phosphatase is not destroyed either by alcohol or by exposure to 56°C in completely dehydrated state, the maximum decrease in activity observed having amounted to less than 20%. Moreover, purification of phosphatase by alcohol precipitation has been successfully used by Martland and Robison.[3] It appeared possible to demonstrate phosphatase in celloidin or paraffin sections on the basis of the following principle: If tissue sections containing active phosphatase are incubated with a solution of sodium glycerophosphate or of some other suitable ester-phosphate, such as hexose-phosphate or nucleinate, at a suitable pH, at the sites where phosphatase is present, PO_4 ions will be split off. These ions may be trapped at the spot by the salts of metals whose phosphates are insoluble. A precipitate of insoluble phosphate forms which, if visible, or made visible, indicates the presence of phosphatase. A useable technic has been worked out on the basis of this principle.

Fixation. Phosphatase is dissolved or destroyed by most of the routine fixatives. In alcohol, however, it is preserved for many weeks at least. Thin slices of fresh tissue should be fixed in 95% alcohol for about one day.

Unfortunately, decalcification is impossible, because all acids destroy phosphatase completely.

Embedding. Both celloidin and paraffin embedding is suitable. If the tissue is well dehydrated, exposure to paraffin at 56 to 60°C for 2 hours will do no harm. Celloidin sections are incubated

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* This work has been done under a grant from the Douglas Smith Foundation for Medical Research of the University of Chicago.

† Manuscript received June 1, 1939.

[1] Berenblum, I., and Chain, E., *Biochem. J.*, 1938, **32**, 295.

[2] Kay, H. D., *Biochem. J.*, 1928, **22**, 855.

[3] Martland, M., and Robison, R., *Biochem. J.*, 1929, **23**, 237.

with the substrate solution without any previous preparation, but paraffin sections must be protected with a thin film of collodion against loss of the enzyme by dissolution. The sections are dipped in a dilute solution (about 0.5 to 1%) of collodion in alcohol-ether. The membrane is hardened subsequently by dipping the section in 90% alcohol.

Incubation. The composition of the substrate fluid can be varied considerably according to the need. As a routine, 2 stock solutions, a 2% solution of sodium glycerophosphate, and a 2% solution of calcium nitrate, both in distilled water, can be used. One part of each of the solutions is added to about 6 to 8 parts of distilled water. In general, no buffering is necessary unless one wants to study the effect of a certain pH on the process. Needless to say, buffers forming insoluble calcium salts cannot be used, such as phosphate or borate buffers. Sodium barbital, acetate, or citrate buffers may be used. Acid media should be avoided because the solubility of calcium phosphate rapidly increases with the decrease of the pH. Any substance whose action on the process is to be tested can be added to the solution except substances which form precipitates with calcium (*e. g.*, fluoride). The time of exposure should be at least 2 hours at 37°C. Some increase in the intensity of the reaction has been observed for as long as 5 hours.

The salts of some other metals besides calcium have been tried to trap the phosphate ions but all of them proved to be much inferior to calcium. The heavy metals, as a rule, greatly inhibit phosphatase activity; others, while apparently indifferent to the enzyme (cobalt, nickel), on long exposure form compounds with the tissue proteins, thus making the selective demonstration of the phosphate precipitate difficult.

After incubation the sections are washed in a dilute solution of calcium nitrate and stored in it until the next step. Distilled water should not be used for this purpose because it may dissolve some of the precipitate.

Visualization of the precipitate. Since calcium phosphate is hardly visible, it is transformed into a colored compound. One of the following methods may be used:

1. Kóssa's method of exposure to a dilute solution of silver nitrate (about 0.5%) in direct sunlight. Calcium phosphate is transformed to silver phosphate. The latter, being photosensitive, will darken in sunlight. The whole reaction takes about 5 to 30 minutes, depending on the intensity of the sunlight. Instead of sunlight, an ultraviolet light source may be used. After the desired color is obtained, the section is rinsed in distilled water and fixed in a dilute hypo solution

(1 to 2%). The sites of phosphatase are shown in a rich golden-brown color.

2. Transfer the section to a dilute solution of cobaltous nitrate (1 to 2%) for about 5 minutes. Rinse thoroughly in several changes of distilled water. Transform cobalt phosphate into black cobalt sulphide by immersing the section in a very dilute solution of yellow ammonium sulphide (a few drops to a Coplin jar) for a few minutes. Wash.

The sections can be counterstained with any stain desired. Besides silver and cobalt the salts of several other metals have been tried but proved inferior to the methods mentioned.

Results. Lack of space precludes description at length of pictures obtained. The main points of interest are: In normal animals belonging to the same species, individual variations of pattern are very slight; there may be more or less marked differences in the phosphatase picture of the same organ in different animal species, though in most cases the type of pattern is closely similar; there is some difference between pictures obtained with different ester-phosphate substrates. The tissues and cells richest in phosphatase are, respectively: The surface epithelium of the small intestine; the transitional epithelium of the urinary bladder; both epithelium and connective tissue of the mammary gland; the convoluted and straight

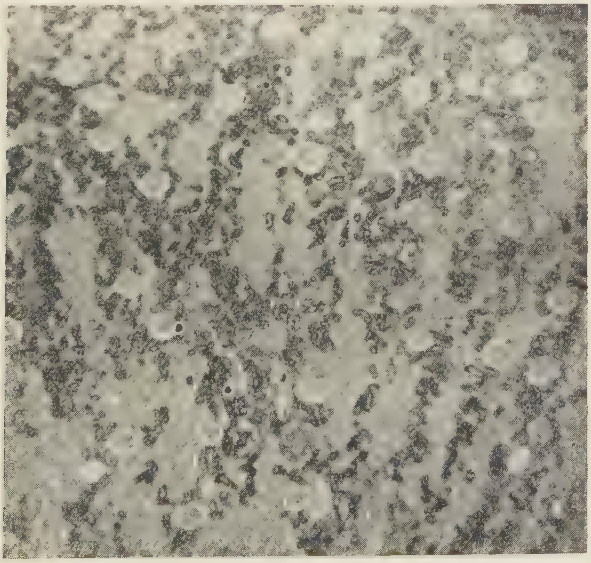

FIG. 1.

Renal cortex of the dog. Proximal convoluted tubules stained black. Magnification: 28 diameters.

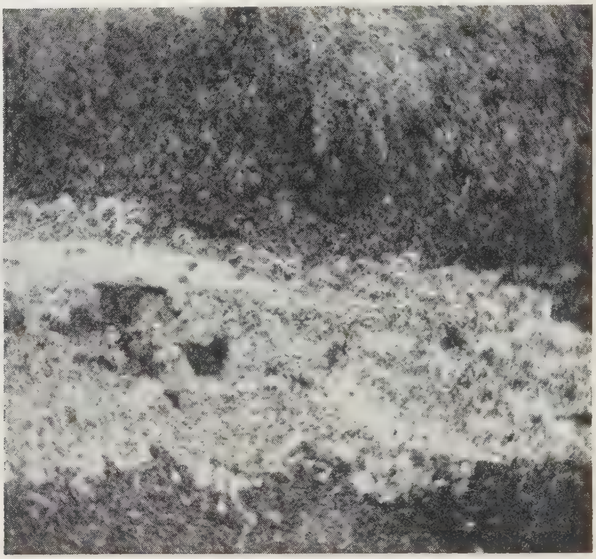

FIG. 2.

Adrenal of the rabbit. Positive reaction (dark) in the cortex, especially in its deeper layers, and in islets of cortex tissue within the medulla. Medulla itself negative. Magnification: 100 diameters.

segments of the proximal convoluted tubules in the kidney; the deep layers of the adrenal cortex; the lining of the seminiferous tubules; ossifying cartilage and embryonic perichondrium; the adventitia of medium-sized arteries, and the endothelium of capillaries in certain organs. No phosphatase at all, or a minimal trace of it, is found in muscle and nervous tissue, stomach mucosa, skin, adrenal medulla, and the glomeruli and all parts of the kidney tubules distal to the transition of the proximal convoluted tubules into Henle's loops. Phosphatase pictures of liver, pancreas, and some other organs show marked differences in various animal species. The number of pathological specimens examined is too small yet to permit comprehensive statements, but the constant absence of phosphatase from young granulation tissue and from malignant epithelial tumor cells deserves mention.

The results of microtechnical demonstration and of chemical determination of phosphatase are in very good agreement.

10787 P

The Sensory Cortex of the Chimpanzee.*

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The location, extent and subdivision of the sensory cortex is not known exactly in any species higher than the macaque.

By local cortical strychninization and clinical observation of the animal the location, extent and subdivision of the macaque's sensory cortex was mapped in 1924.[1] By local cortical strychninization and recording of the electrical activity of the leg, arm and face sensory thalamic nuclei, which had been diagnosed physiologically,[2] the original findings were confirmed.[3] Finally, by local strychninization of the cortex this time combined with recording of the typical and typically distributed changes in the electrical activity of the cortex itself (strychnine-spikes) the functional organization of the macaque's sensory cortex was delineated in detail.[4, 5]

This functional organization is so characteristic that it should be recognizable in higher species, such as the chimpanzee, even if details there should prove to be different.

The methods initially used in the macaque would be dangerous with an animal as strong as an anthropoid ape. The sensory thalamic nuclei have not as yet been diagnosed physiologically in the anthropoid and to do it would be equally dangerous. We have, therefore, employed the third procedure, that of local cortical strychninization combined with the recording of the electrical activity of the cortex itself; this can be done in the fully anesthetized animal. When this combination of methods is utilized to study the functional organization of the chimpanzee's cortex it discloses a large region the functional organization of which is comparable to that of the sensory cortex of the macaque. The obvious inference is that the region so disclosed is sensory.

Thus far 4 chimpanzees' brains have been so investigated with

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* Aided by a grant from the John and Mary R. Markle Foundation.

[1] Dusser de Barenne, J. G., *Proc. Roy. Soc. London*, 1924, B. **96**, 272.

[2] Dusser de Barenne, J. G., and Sager, O., *Arch. Neurol. Psychiat.*, Chicago, 1938, **38**, 913.

[3] Dusser de Barenne, J. G., and McCulloch, W. S., *J. Neurophysiol.*, 1938, **1**, 176.

[4] Dusser de Barenne, J. G., and McCulloch, W. S., *J. Neurophysiol.*, 1938, **1**, 69.

[5] Dusser de Barenne, J. G., McCulloch, W. S., and Ogawa, T., *J. Neurophysiol.*. 1938, **1**, 436.

essentially similar results. However, the variation in the convolutions of the brain of this species is so great that it seems inadvisable to give a composite diagram. We have, therefore, represented in the accompanying figure the sensory cortex on the outer surface of one hemisphere of that brain from which most information was gathered. On this chimpanzee 35 local strychninizations were performed and in all cases the electrical activity of five loci on the cortex was recorded simultaneously. During the disturbances induced by the strychnine the electrodes were frequently moved to different loci so that the information obtained is unusually complete and comprises upward of 200 observations on this one brain.

The question marks in the diagram indicate the only remaining uncertainties in this particular brain. Since these questionable parietal areas responded differently from the immediately adjacent areas of the sensory cortex, and since they are homologous to the supramarginal and angular gyri in man, they probably do not belong to the sensory cortex proper. No data are available about the little

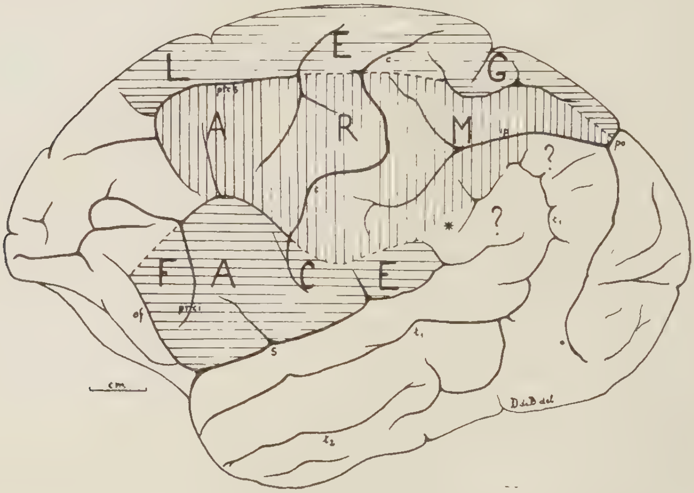

FIG. 1.

Sensory cortex of the chimpanzee. This figure represents as accurately as is possible in one plane the outer surface of one hemisphere of chimpanzee No. 4. The precentral boundary between the face and arm subdivisions lies in this hemisphere unusually low, but was verified both by electrical stimulation with motor response and with the strychnine method. Unusual is also that this boundary is marked by a definite sulcus across the precentral gyrus. The figure schematizes the results of over 200 observations on this one brain.

triangular area marked * to indicate whether it belongs to the arm or face subdivision. Most striking is that, in broad outline, the sensory cortex of the chimpanzee is essentially similar to that of the macaque and occupies such a large area on the outer surface of the hemisphere.

10788 P

Recovery of Carbon-Monoxide Poisoned Rats After X-ray Treatment.*

JOHN A. CAMERON. (Introduced by A. G. Hogan.)
From the Department of Zoology, University of Missouri.

It has been shown that illumination with visible light removes the effect of carbon-monoxide poisoning in small, transparent organisms which absorb the poison from water solutions.[1] This report concerns the effect on larger, more opaque, air breathing organisms, of a more penetrating type of radiation—the X-ray. Acknowledgments are gratefully made to Dr. L. J. Stadler, of the U. S. D. A. and the Department of Field Crops, University of Missouri, for use of the X-ray equipment and services of an operator.

Albino rats descended from Wistar stock were used; each experiment consisted of one treated and one control animal. The 2 animals were of the same age and sex and not more than 10% difference in weight was allowed. The 2 negative cases reported were cases where great difference in weight existed.

Carbon monoxide was generated by dropping formic acid into hot sulphuric acid and collected by displacement of water in storage bottles.[2] To each bottle was added 5% of oxygen from a commercial cylinder. The gas mixture was administered to the rats by passing it into a tightly covered glass jar under pressure of a constant head of water in an elevated funnel opening into the gas bottle. Each experiment was conducted as follows. The 2 rats were placed simultaneously in the gas chamber and the gas admitted. Two or 3 observers watched through the sides of the gas chamber to note any differences in apparent susceptibility and to determine when both rats seemed to have stopped breathing. The gas being shut off, the rats were simultaneously removed and laid on their backs in similar glass battery jars of 9-inch diameter. Each jar was covered with 2 sheets of wet paper toweling and the jar containing the rat adjudged to be the weaker and showing the smaller signs of recovery was placed under the X-ray tube which had already been "warmed up" by its operator. The Victor machine used supplied 123 r per minute with no filter except the paper toweling, running at 165 K. V. P. on a tube current of 4 ma., and at 12 inches target distance. Exposures of 5 to 10 minutes were made in each experiment. Recovery was judged to have taken place when the animals behaved in a normal manner on the day following the experiment and remained apparently normal for 3 days thereafter.

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* This study was aided by a grant from the Research Council of the University of Missouri.

[1] Warburg, Otto, and Negelein, Erwin, *Biochem. Z.*, 1928, **193**, 339.

[2] Fisher, K. C., and Cameron, John A., *J. C. and C. Physiol.*, 1938, **11**, 433.

The 41 experiments performed on pairs of rats from 8 days to 18 months old yielded results of all the 4 possible types.

In 3 cases the carbon monoxide administered was insufficient to kill either rat, both animals recovering.

In 21 cases the animals were "over-gassed" and neither rat recovered.

Two of the earliest experiments matched X-rayed rats weighing 32 and 44 g against control rats weighing 52 and 66 g. Here the controls recovered and the X-rayed rats died. In subsequent experiments not over 10% difference in weight was allowed.

Fifteen experiments ended in recovery of the X-rayed rat and the death of the control in spite of the fact that the least promising member of the pair was always chosen for X-ray treatment. In 5 of these cases the X-rayed rat had recovered sufficiently to rise to its natural quadrupedal position by the time the exposure was over and the X-ray compartment opened.

It is concluded that X-ray treatment applied to the surface of the body in amounts readily tolerated by the subject was the critical factor determining recovery in these cases.

10789 P

Carotid Body Reflexes in the Dog.

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In vagotomized dogs anesthetized with morphine and chloralose the following results have been obtained:

1. Contrary to our previous belief[1, 2] carotid chemoreceptors of some of these animals were continuously active under normal experimental conditions because—

(a) Respiration was often momentarily depressed when both sinus nerves were inactivated by cooling or by injection of procaine. This occurred also in decerebrated animals or anesthetized dogs breathing oxygen, and when rise in blood pressure upon blocking the nerves was obviated by preliminary selective denervation of the pressure receptors.

(b) Respiration was temporarily depressed when the fluid perfusing both carotid bodies was cooled from 38° to 26°C or less (confirming Bernthal and Weeks[3]). Similar results were obtained when the perfusing fluid was phosphate-buffered Locke's solution at pH 7.5, free of CO_2 (less than 1 mm tension) and saturated with O_2 (more than 150 mm tension)—in brief, when the carotid chemoreceptors were subjected to much smaller amounts of the materials known to stimulate them than could ever be the case under physiological conditions. Part or all of the continuous activity revealed by these experiments may therefore have been contributed by something other than ordinary chemoreceptors; temperature receptors are suggested as a possibility.

2. The sensitivity of the carotid chemoreceptors was tested by perfusion of both carotid bodies with oxygenated Locke's solution of which either pH was kept constant and CO_2 tension was varied (according to the Hasselbalch equation) or CO_2 was removed and the pH varied by means of phosphate buffers. In those animals whose carotid bodies showed unusually great reactivity, the following results were obtained:

(a) *CO_2 tension:* The smallest change by which breathing was reflexly affected was 5 mm Hg, which was effective in one trial out

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[1] Comroe, J. H., Jr., and Schmidt, C. F., *Am. J. Physiol.*, 1938, **121**, 75.

[2] Schmidt, C. F., *Macleod's Physiology in Modern Medicine*, 8th ed., pp. 505-516, St. Louis, 1938.

[3] Bernthal, T., and Weeks, W. F., *Am. J. Physiol.*, 1939, **127**, 94.

of 8 on 5 animals. Changes of 10 mm were effective in 4 out of 8 trials in 4 animals. In all these cases the respiratory effects were slight (changes of 8-21% in minute volume). Changes of 15 mm were tested 22 times (in 8 animals) but only 8 tests gave positive results. Only at a 20 mm change were the results marked and consistent (11 positive results in 11 trials in 4 animals). In interpreting these results we realize that anesthesia, trauma, artificial perfusion, etc., all tended to reduce the sensitivity of the carotid bodies and that the most sensitive preparation might therefore be regarded as closer to the intact state than the others. Yet there are undoubtedly wide natural variations in the sensitivity of the chemoreceptors; this is revealed in the variability in normal men of the hyperpnea of anoxemia, which is essentially a chemoreceptor phenomenon. Choosing between these alternatives, we believe our results to indicate that in the average dog, under our experimental conditions, the smallest alteration in CO_2 tension that will change chemoreceptor activity sufficiently to produce a significant respiratory response, is probably closer to 20 mm than to 10 mm. An occasional response to a 5 mm change seems to represent exceptional reactivity, not that of the average animal.

(b) *Acidity:* Changes of 0.1 pH unit in either direction produced distinct though slight respiratory effects in 4 of 7 tests in 4 animals. Changes of 0.15 elicited greater and more consistent effects, 0.2 still more so. Changes smaller than 0.1 were not tried.

3. The threshold of the respiratory center of the dog under these conditions (vagotomy, morphine-chloralose anesthesia) has been tested by noting the change in CO_2 tension of arterial blood when respiration began to be stimulated distinctly during rebreathing of oxygen. In the experiments so far performed, this has been an increase of 2 to 3 mm in CO_2 tension; there were no changes in pH detectable by the glass electrode. After denervation of both carotid bodies these results were unchanged.

Although the carotid bodies of these animals showed greater sensitivity to changes in CO_2 tension and pH than any previously reported, the conclusion of our earlier experiments—that under all ordinary conditions the chemical control of breathing can be accomplished by the cells of the center without involving the relatively insensitive chemoreceptors—is reaffirmed.

10790

Heparin and Plasma Albumin in Relation to Thromboplastic Action of Trypsin, Cephalin and Brain Extracts.

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Evidence has recently been presented for the existence of a *thromboplastic enzyme* factor in blood clotting.[1] Its rôle is to mobilize cephalin and calcium for thrombin formation.[2] With recently standardized technical procedures,[3] observations have been extended to include the known normal antithrombic factors.

The degree to which different amounts of heparin can inhibit clotting of citrated dog plasma by crystalline trypsin, under varying conditions of calcium and cephalin mobilization, is evident in the controlled experiments of Table I.

Sufficient heparin can greatly delay coagulation even under conditions most favorable to the action of trypsin, namely, abundance of added cephalin and calcium. Nevertheless, clotting eventually occurs in all tubes. The heparin inhibition varies inversely with the clot-aiding efficacy of the thromboplastic mixture.

Table II shows the varying extent of "antiprothrombic" action of heparin during the formation of thrombin from recalcified prothrombin in the presence of (1) aqueous brain extract,* (2) its equivalent of isolated phospholipids, (3) a comparable solution of cephalin, (4) crystalline trypsin.

The heparin is able completely to prevent thrombin formation in the presence of cephalin and (practically) in the case of the slightly more potent solution of isolated P-lipids. With whole brain extract there is delayed activation (confirmed) only evident in the first minute of incubation. Thereafter, the thrombin formed in the presence of heparin is as potent as in the control. Trypsin behaves very similarly to the crude thromboplastin, namely, evincing some delay in the early minutes of prothrombin activation. The occurrence of a minor degree of the same phenomenon in the control (*sans* heparin)

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[1] Ferguson, J. H., and Erickson, B. N., PROC. SOC. EXP. BIOL. AND MED., 1939, **40**, 625.

[2] Ferguson, J. H., and Erickson, B. N., *Am. J. Physiol.*, 1939, **126**, 661.

[3] Ferguson, J. H., *J. Lab. and Clin. Med.*, 1938, **24**, 273.

* Thromboplastin prepared from a dry brain preparation kindly supplied by Dr. A. J. Quick. Solution analysed: Total P-lipid = 11 mg. incl. Cephalin = 4.6 mg; Protein = 104 mg %.

TABLE I.
Effects of Varying Concentrations of Heparin upon Clotting of Citrated Dog Plasma by Crystalline Trypsin, Cephalin, and Calcium.

	Trypsin (mg in 0.25 cc)	Dist. water (cc)	Cephalin (cc of 1:1000)	N/10 $CaCl_2$ (cc)	Heparin* (mg in 0.25 cc)	Cit. Plasma (cc)	Clotting-time (38°C)
1.	—	.75	.25	—	—	1	No clot
2.	—	.75	—	.25	—	1	325 sec
3.	—	.5	.25	.25	—	1	100 ”
4.	—	.5	—	.25	0.05	1	1340 ”
5.	—	.5	—	.25	0.1	1	No clot
6.	—	.5	—	.25	1.0	1	” ”
7.	—	.25	.25	.25	0.05	1	240 sec
8.	—	.25	.25	.25	0.1	1	375 ”
9.	—	.25	.25	.25	1.0	1	No clot
10.	.25	.75	—	—	—	1	>2<7 hr
11.	.25	.5	.25	—	—	1	90 sec
12.	.25	.5	—	.25	—	1	58 ”
13.	.25	.25	.25	.25	—	1	22 ”
14.	.25	.5	—	—	0.05	1	>2<7 hr
15.	.25	.5	—	—	0.1	1	>2<7 ”
16.	.25	.5	—	—	1.0	1	>7<15 ”
17.	.25	.25	.25	—	0.05	1	>2<7 ”
18.	.25	.25	.25	—	0.1	1	>2<7 ”
19.	.25	.25	.25	—	1.0	1	>7<15 ”
20.	.25	.25	—	.25	0.05	1	135 sec
21.	.25	.25	—	.25	0.1	1	2 hr
22.	.25	.25	—	.25	1.0	1	>7<15 ”
23.	.25	—	.25	.25	0.05	1	29 sec
24.	.25	—	.25	.25	0.1	1	45 ”
25.	.25	—	.25	.25	1.0	1	>2<7 hr

*Ordinary commercial heparin (H., W. and D.'s.)

TABLE II.

Effects of Heparin on the Formation of Thrombin from Recalcified Prothrombin in the Presence of Various Thromboplastic Agents.
Thrombic mixture (T) = 4 cc prothrombin soln. + 1 cc activator(s) *plus* inhibitor (suitably diluted), incubated at 15°C. All mixtures include $CaCl_2$ (0.05 m.eq. Ca per 5 cc T). Cephalin, trypsin, and heparin : mg per 5 cc T. Clotting times (sec) at 38°C : 1 cc fibrinogen (prothrombin-free) + 0.5 cc T.

T	Thromboplastic agent	Inhibitor	Thrombin formation period, *minutes*, at 15°C)					
			1′	5′	10′	20′	30′	60′
1.	†Cephalin (0.1)	—	—	50″	20″	11″	9″	9″
2.	,, ,,	Heparin (0.5)	—	∞	∞	∞	∞	∞
3.	‡Brain P-lipids (0.025)	—	50″	20″	10″	8″	6″	6″
4.	,, ,, ,,	Heparin (0.5)	∞	∞	∞	∞	*1800″	*360″
5.	Thromboplastin (0.25 cc) incl. 0.025 mg P-lipid	—	35″	16″	9″	7″	7″	7″
6.	,, ,, ,, ,, ,, ,, ,,	Heparin (0.5)	*720″	16″	10″	7″	7″	7″
7.	Trypsin (0.125)	—	∞	30″	15″	12″	11″	8″
8.	,, ,,	Heparin (0.5)	∞	97″	32″	13″	11″	9″
9.	,, (0.05)	—	∞	95″	30″	15″	14″	11″
10.	,, ,,	Heparin (0.5)	∞	∞	660″	70″	32″	18″

*Indicates incomplete coagulation.

†The cephalin was an old preparation which appears to have lost somewhat more than three-fourths of its original potency.

‡Quick's "thromboplastin" extracted with alcohol-ether (3:1); acetone-insoluble P-lipids recovered in ether-petroleum ether, dried, and prepared in aqueous solution (1:10,000).

may be evidence for an enzyme inhibitor in the prothrombin preparation.

Fresh tissue extracts have a much greater thromboplastic potency than can be accounted for on the basis of their phospholipid content. There is some evidence for this even in the present experiment in which the aqueous extract has been kept for 3 days in the ice-box, pending analyses.

These data afford additional evidence that the close analogy of thromboplastin and trypsin extends to their behavior in the presence of heparin. The antiprothrombic action of heparin is so brief in these cases, as compared with tests using isolated P-lipids, that it is clear how Quick[4] and Brinkhous, Smith, *et al.,*[5] missed the classical effect.[6]

Antithrombic actions of heparin and albumin. Table III confirms Quick's[7] finding of the synergism of the otherwise-negligible "antithrombic" actions of heparin and plasma *albumin* (the dialysed residue after $(NH_4)_2SO_4$ pptn. of plasma globulins), with one important exception. There is no evidence of any *progressive* inactivation of thrombin, although tests were made on thrombic mixtures of the 4 types shown in Table II and with the addition of (1) albumin, (2) heparin, and (3) albumin plus heparin. The phenomena of Table III, significantly, were reproducible with *each type* of thrombic mixture.

Conclusions. There is no valid reason to suppose that the fundamental process of thrombin formation differs as between the phospholipid and the enzymic modes of activation.[2] The fact of the

TABLE III.
Synergism Between Antithrombic Actions of Heparin and Plasma Albumin.
Thrombin = 4 cc prothrombin soln. + 0.5 cc cephalin (1:500) + 0.5 cc $CaCl_2$ (N/10), aged for over 2 hours but stabilized by low temperature (10°C).

	Thrombin (cc)	Dist. Water (cc)	Heparin (mg in 0.25 cc)	Albumin (cc)	Fibrinogen* (cc)	Clotting-time (38°C)
1.	.5	.75	—	—	1	15 sec
2.	.5	.5	.05	—	1	20 ”
3.	.5	.25	—	.5	1	37 ”
4.	.5	—	.05	.5	1	25 *min.*
5.*	.5	—	.05	.5	1	24 ”

*Fibrinogen added exactly 1 minute after last anticoagulant, except in 5 where the heparin, albumin and thrombin were allowed to interact for 10 minutes.

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[4] Quick, A. J., *Am. J. Physiol.*, 1936, **115**, 317.

[5] Brinkhous, K. M., Smith, H. P., Warner, E. D., and Seegers, W. H., *Am. J. Physiol.*, 1939, **125**, 683.

[6] Howell, W. H., and Holt, E., *Am. J. Physiol.*, 1918, **47**, 328.

[7] Quick, A. J., *Am. J. Physiol.*, 1938, **123**, 712.

significant quantitative differences in the cited tests shows that the so-called "antiprothrombic" effect of heparin is not to be interpreted as an alteration of the prothrombin itself, but rather as a manifestation of antagonisms between the heparin and the thromboplastic factors. There is an experimental difference between the simple addition of cephalin and its enzymic mobilization from protein-phospholipid combinations.

The results of all clotting experiments must be evaluated with reference to (1) quantitative relationships between the various factors, (2) the question of cephalin mobilization, and (3) time factors involved in thrombin formation, the thrombin-fibrinogen interaction, and in the overcoming of the inhibitory mechanisms. It is significant that thromboplastic enzyme has demonstrable powers of overcoming the "antiprothrombic" inhibitory mechanisms. The "antithrombic" action of heparin-albumin mixtures is immediately evident and is not dependent upon the type of thromboplastic agent used in the preparation of the thrombin.

10791

The Sugar Tolerance of Alcoholic Patients.

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In previous publications from these laboratories[1, 2] we have reported studies on the effect of glucose on the oxidation of alcohol. We found that the oxidation of alcohol *in vitro* was accelerated when glucose was simultaneously oxidized.[1] Studies on the effect of glucose on the oxidation of alcohol in man revealed that there was a moderate increase after glucose administration, and a more marked increase after glucose and insulin.[2] It is well known that alcoholic patients eat very little while on a drinking debauch, and it was suggested that the difference between the effect of the glucose and the glucose plus insulin might be due to an impaired ability of the patients to oxidize the added glucose without insulin because of their previous low carbohydrate intake.

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[1] Goldfarb, W., and Bowman, K. M., PROC. SOC. EXP. BIOL. AND MED., 1938, **39**, 471.

[2] Goldfarb, W., Bowman, K. M., and Parker, S., *Am. J. Physiol.* (Proc.), 1939.

It is well known that undernutrition and a lowered carbohydrate intake diminishes the ability of the organism to oxidize glucose. Chambers[3] has recently reviewed the literature on the effect of undernutrition and has shown that it results in an impaired tolerance to glucose. We have attempted to estimate this impairment of carbohydrate tolerance in alcoholic patients by observations of the sugar tolerance curves.

Patients were chosen from among those admitted between 4 P. M. and midnight to eliminate the effect of any changes in the dietary. The first tolerance test was performed the next morning before breakfast, and was repeated again one week later after the patients had been maintained on a routine hospital diet. In 9 cases the tolerance was tested with a single dose of 50 g of glucose by mouth, and in the remaining 9 cases we used the 2-dose test with 20 and 40 g of glucose described by Jolliffe.[4]

Results. The blood sugar curves obtained with the 2-dose test are presented in Table I. The average curve of the 9 patients on admission showed an increased blood sugar after the 20 g dose of 42 mg %, and the 40 g dose of glucose caused a further rise of 38 mg %. The 2½-hour specimen was higher than the fasting specimen. The average blood sugar curve obtained after one week in the hospital approached the response of normal patients. The response to the 20 g dose was 15 mg %, and the blood sugar curve following the 40 g dose was only 14 mg % higher than the ½-hour specimen. For comparison the average blood sugar curve of 10 normal subjects observed by Jolliffe is appended. In each of the individual cases there was an improved tolerance after the hospital stay.

In a similar fashion the average tolerance curve of 9 patients tested with 50 g of glucose was improved after one week in the hospital. In 7 of these cases (10, 11, 13, 14, 16, 17, 18) tolerance was improved. Patient No. 12 showed a questionable change, while patient 15 had a definitely diminished tolerance.

In 16 of the 18 patients examined there was an increase in the sugar tolerance after one week in the hospital. The improvement may be attributed to either or both of the following reasons: (1) the patient became acclimated to the surroundings and was calmer emotionally; or (2) an improved dietary regime may correct the effects of undernutrition in the patient. There have been a number of conflicting reports in the literature on the rôle of the emotional

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[3] Chambers, W. H., *Phys. Rev.*, 1938, **18**, 248.

[4] Jolliffe, N., *J. Clin. Invest.*, 1930, **9**, 363.

TABLE I.

Glucose Tolerance Tests with 20 g of Glucose *per os*, followed with 40 g after ½ hr (Jolliffe).

Patient	Test on admission						Test after 1 week in hospital					
	Fasting	½ hr	1 hr	1.5 hr	2 hr	2.5 hr	Fasting	½ hr	1 hr	1.5 hr	2 hr	2.5 hr
1	103	149	167	121	129	140	91	104	116	111	83	55
2	103	130	154	160	136	120	79	111	146	97	—	53
3	123	222	222	196	118	100	101	118	108	97	98	98
4	80	89	109	166	119	109	85	95	90	86	76	81
5	88	116	131	113	109	100	74	99	84	74	70	70
6	98	128	217	160	120	91	100	115	169	147	124	96
7	75	152	189	233	164	143	108	116	154	136	104	71
8	78	111	172	139	100	77	112	119	138	125	105	99
9	105	139	227	250	179	128	91	111	117	109	101	89
Avg	95	137	175	171	130	112	93	110	124	109	95	79
Age of 10 normal cases (Jolliffe)							90	102	100	95	87	80

TABLE II.

Glucose Tolerance Tests with 50 g of Glucose *per os*.

Patient	Test on admission					Test after 1 week in hospital				
	Fasting	½ hr	1 hr	2 hr	3 hr	Fasting	½ hr	1 hr	2 hr	3 hr
10	83	218	234	93	75	97	166	165	133	64
11	104	192	234	200	130	77	156	133	131	92
12	100	250	133	80	75	100	190	178	140	105
13	97	158	186	75	80	75	146	74	70	77
14	135	206	208	190	156	88	143	135	91	—
15	80	143	168	75	—	96	181	217	100	—
16	77	127	138	147	136	98	127	140	100	79
17	96	110	196	126	120	84	115	130	91	87
18	129	185	189	146	129	107	137	101	81	80
Avg	100	177	187	126	106	91	151	141	104	82

effect on the sugar tolerance curve. Schwab[5] and Jacobi and Koritter[6] studied neurotic patients in various emotional moods and found normal sugar tolerance curves. Mann,[7] Craig,[8] and Katzenelenbogen, *et al.*,[9] observed a delayed curve in a large percentage of neurotic patients. Diethelm[10] found that acute emotion caused a sharp rise of the tolerance curve, with a rapid fall, and the peak of the curve was roughly proportional to the intensity of the emotion. Henry and Mangam[11] found a diminished tolerance to glucose in psychotic patients during a depressive phase of activity, while during the excited phase of the psychosis the tolerance was increased. None of the patients we tested were depressed on admission, while the majority were tremulous and tended to be excited. The symptoms of excitement were absent when the patients were reëxamined one week later. It is difficult to evaluate the effect of the emotional effect in these patients, but it seems probable that the tolerance on admission was diminished despite the tendency to excitement.

It is generally agreed, however, that undernutrition results in a diminution of the sugar tolerance. This diminution has been observed both clinically and experimentally, and the extensive literature has been admirably reviewed by Chambers.[3] The dietary regime of alcoholic patients has frequently been described as inadequate both in quantity and quality, and it is suggested that the diminished glucose tolerance observed in these patients is largely due to the dietary deficiency. This contention is supported by the fact that adequate diet for one week resulted in a more normal sugar tolerance curve.

Summary and Conclusions. The sugar tolerance was studied in a group of 18 alcoholic patients. On admission there was a marked diminution of the sugar tolerance, which improved after one week in the hospital on a normal diet. It is suggested that the diminished tolerance was due to an undernourished state previous to admission.

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[5] Schwab, S. I., *Arch. Neurol. Psych.*, 1922, **8**, 401.

[6] Jacobi, J., and Koritter, H., *Psychiat. Neurol. Wchnschr.*, 1923, **34**, 1.

[7] Mann, S. A., *J. Mental Sc.*, 1925, **71**, 443.

[8] Craig, R. N., *Lancet*, 1927, **1**, 925.

[9] Katzenelenbogen, S., and Friedman-Buchman, E., *Am. J. Psych.*, 1933, **13**, 321.

[10] Diethelm, O., *Arch. Neur. Psych.*, 1936, **36**, 342.

[11] Henry, G. W., and Mangam, E., *Arch. Neurol. Psych.*, 1925, **13**, 743.

10792

Metabolism of the Tubercle Bacillus in Long's Synthetic Medium.*

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The rapid growth of mycobacteria in media which contain glycerol was first noted by Nocard and Roux.[1] The growth characteristics of the tubercle bacillus in such media have since been studied by many investigators. Synthetic media, similar to that of Long,[2] are now extensively used in studies of the metabolism of mycobacteria. Long and Finner[3] demonstrated a direct relation between the initial concentration of glycerol and the maximum weight of bacilli which could be obtained in such a medium. Dingle and Weinzirl[4] determined the nitrogen metabolism and made approximate analyses of the glycerol content. Similar determinations were made by Wedum.[5] As yet no accurate measurement has been made of the consumption of glycerol and its relation to the mass of bacilli. Such data are given in the present communication.

Exactly 200 cc of sterile Long's synthetic medium,[2] with 6% of glycerol, were pipetted into sterile tall round bottles of 1 liter capacity. The bottles were loosely stoppered with cotton to facilitate exchange of gases. The bottles were placed on their side; they were inoculated and then incubated at 37.5°C.

At the end of each week one inoculated bottle and one uninoculated bottle were treated as follows: Exactly 50 cc of the clear fluid below the mass of bacilli at the surface were run into 5 cc of 1.0 N H_2SO_4.[6] The bacilli were then filtered through a tared Gooch crucible, washed with cold water, and dried to constant weight at a low temperature. An excess of acid mercuric nitrate was added to a measured volume of the acidified sample, alkali was added until the reaction became neutral, and the volume was then brought to the mark. An aliquot of the filtrate was treated with $CuSO_4$ and

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* This study was aided by the Bartlett Memorial Fund and by the Douglas Smith Foundation for Medical Research of the University of Chicago.

[1] Nocard and Roux, *Ann. Inst. Past.*, 1887, **1**, 19.

[2] Long, E. R., and Seibert, F. B., *Am. Rev. Tuber.*, 1926, **13**, 393.

[3] Long, E. R., and Finner, L. L., *Am. Rev. Tuber.*, 1927, **16**, 523.

[4] Dingle, J. H., and Weinzirl, J., *J. Bact.*, 1932, **23**, 281.

[5] Wedum, A. G., *J. Bact.*, 1936, **32**, 599.

[6] Friedemann, T. E., *J. Bact.*, 1938, **35**, 527.

an excess of $Ca(OH)_2$. The final filtrate was then oxidized with alkaline $KMnO_4$ as described by Freeman and Friedemann,[7] for the determination of glycerol.

It should be noted that all of the "blank" or uninoculated bottles and only one of the inoculated bottles, namely the one which was analyzed after one week of incubation, gave a precipitate immediately after addition of the mercury reagent. Such a precipitate is given by citric acid and by asparagine, both of which are constituents of the synthetic medium. This result indicates a rapid consumption of these constituents.

The results from an experiment with Strain 119 are given in Fig. 1. The dry weight of bacteria and the glycerol consumption increased rapidly after the first week of incubation. The glycerol was completely consumed at the end of about 3.5 weeks. The maxi-

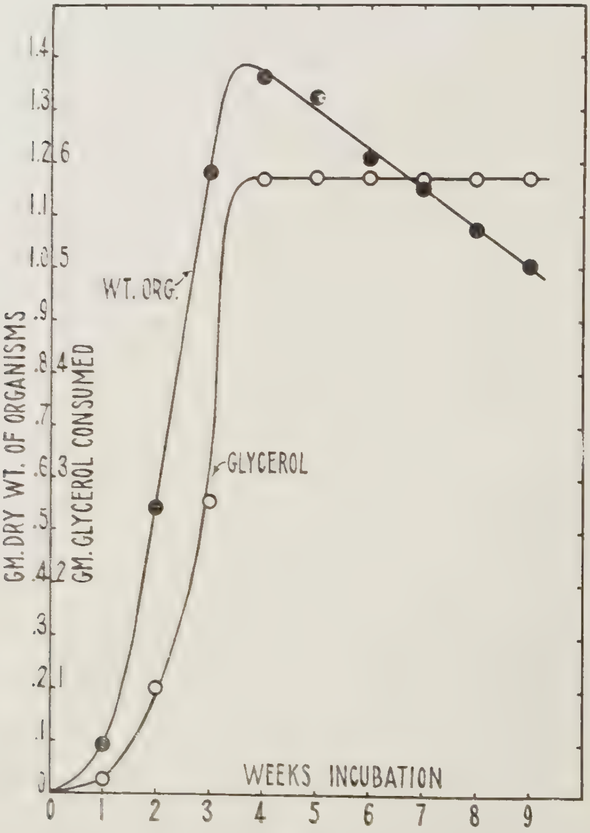

FIG. 1.

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[7] Freeman, S., and Friedemann, T. E., *J. Biol. Chem.*, 1935, **108**, 471.

mum weight of dry bacilli, 1.37 g per 100 cc of culture medium, was obtained at about this time. Thereafter the weight of organisms steadily diminished, due to rapid autolysis. The point at which autolysis was first noted corresponds with the time at which considerable quantities of tuberculin are usually found in the medium.

Quantitative determinations of lactic acid[8] failed to reveal even traces in any of the cultures.

Conclusions. It is shown (1) that the rapid growth of the microorganisms is directly related to the consumption of glycerol and other constituents of the synthetic medium, and (2) that rapid autolysis occurs after exhaustion of the nutrients.

10793 P

Capillary Permeability in the Skin of the Rabbit.*

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The mechanism by which capillaries permit dyes and particulate matter to escape in increased amounts is not clearly understood. Many investigators have observed certain physiological and pathological changes in experimental animals in which the capillaries were more permeable than normal. Burrows[1] and Menkin[2] have stated that trypan blue and India ink when injected intravenously localize and concentrate in areas of inflammation. The latter has also shown that such substances when injected into areas of inflammation are retained longer than when they are injected into normal tissue.

Recent studies in this laboratory have shown that trypan blue and India ink do not always concentrate in areas of inflammation produced by the local application of xylol in the skin of rabbits. These substances concentrate in such areas only when they are injected into the circulation immediately or within a period of less than 5 hours following the application of the xylol. The fact that trypan blue and carbon particles fail to concentrate in xylol-treated areas

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[8] Friedemann, T. E., and Graeser, J. G., *J. Biol. Chem.*, 1933, **100**, 291.

* Aided by grant from the Division of Medical Sciences, Rockefeller Foundation.

[1] Burrows, H., *Localization of Disease*, Wm. Wood and Co., 1932, New York.

[2] Menkin, V., *Physiol. Rev.*, 1938, **18**, 366.

of skin is very interesting since these areas of skin after 5 hours show all the macroscopic and microscopic changes associated with inflammation.

The rabbits were carefully shaven 24 hours or longer before they were used in these experiments. Areas of skin on the sides of these animals were outlined in India ink. Xylol was carefully applied to these areas with a cotton swab. Care was taken not to massage the skin during the application of the irritant. Frequently the xylol diffused along the surface of the epithelium outside the areas marked out with the ink. The skin became blanched immediately following the application of xylol and within a period of less than a minute thereafter it became hyperemic. The areas apparently became edematous within 10 minutes following the application of the irritant. Following an intravenous injection of trypan blue in a 0.2% saline solution the dye localized in the area of skin where xylol was last applied, usually within an interval of 5 minutes or less. The amount of dye that localized in the xylol-treated areas of skin decreased with an increase in the interval between the application of the irritant and the injection of the dye during a period of approximately 2 hours. After this time essentially the same quantity of trypan blue localized in the treated and untreated areas of skin.

Dr. Ralph D. Cressman and I recently observed that rabbits anesthetized with either alcohol or ether failed to show a localization of trypan blue in areas of skin treated with xylol within the same time as the normal animals. The dye appeared first in the areas of skin treated with xylol 15 minutes before the dye was injected rather than in the last areas as observed in the normals. The time that trypan blue first appeared in the xylol-treated areas of skin in the normal rabbits did not occur in every animal as described above, however, the variation in the anesthetized and non-anesthetized rabbits did occur in a sufficient number of animals apparently to be significant.

Staphylococcus antitoxin, diphtheria antitoxin and a saline suspension of vaccine virus each have been found to localize in areas of inflammation produced by xylol when given intravenously immediately following the application of the irritant. There is no concentration of either the antitoxin or the virus in the areas of irritation if they are injected intravenously 24 hours following the application of the xylol.

The observation that capillaries show an increase in permeability for antitoxin is significant from a therapeutic standpoint. Medicinal preparations given intravenously may be concentrated in the tissue by increasing the capillary permeability through the use of counter irritants. Neoprontosil has been observed to localize and concentrate in areas of the rabbits' skin when it was given intravenously immediately following the local application of xylol. A complete study will subsequently be made on the localization of blood-borne materials in areas of inflammation.

The indications are from my observations that increased capillary permeability is of brief duration following injury where xylol is applied and apparently is a phenomenon distinct from that by which leucocytes reach the area of inflammation. The two phenomena however frequently occur together.

The results obtained in these experiments on the relation between the time of application of xylol to the skin of rabbits and the time of localization and concentration of trypan blue following an intravenous injection are similar to the observations of Kline and Winternitz.[3] The latter investigators found that when pneumonia was produced in rabbits with vital stain in their circulation, the involved lung had a uniform blue color; if, however, the dye was injected sometime after the pneumonia developed (20-65 hours) pale gray consolidated areas occurred in the otherwise densely blue stained pneumonic lobes.

10794 P

In vitro Incorporation of Fatty Acids in Phospholipids of Intestinal Mucosa.*

RICHARD H. BARNES, ELMER S. MILLER AND GEORGE O. BURR.

From the Departments of Physiology and Botany, University of Minnesota, Minneapolis.

Sinclair,[1] using elaidic acid, found that phospholipids of the intestinal mucosa after feeding fat, contained some of that fed fat. Perlman, Rubin, and Chaikoff[2] later showed that fed, radioactive phosphorus also became incorporated in the phospholipids of the

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[3] Kline, B. S., and Winternitz, M. C., *J. Exp. Med.*, 1915, **21**, 311.

* Aided by grants from the Rockefeller Foundation and the National Live Stock and Meat Board.

[1] Sinclair, R. G., *J. Biol. Chem.*, 1929, **82**, 117.

[2] Perlman, I., Rubin, S., and Chaikoff, I. L., *J. Biol. Chem.*, 1937, **122**, 169.

intestinal mucosa. Both of these findings have been repeatedly confirmed. Robinson, *et al.*,[3] have also shown that slices of intestinal mucosa incubated in a Warburg apparatus with radioactive phosphorus would bring about an incorporation of radioactive phosphorus in the mucosal phospholipids. Using the conjugated, unsaturated fatty acids of corn oil as a tagged fat[4] the *in vitro* incorporation of fatty acids in the intestinal mucosal phospholipids has been studied.

Male, albino rats were anesthetized with ether and their abdomens opened. Three cc of an emulsion containing bile salts, lipase (pancreatin), water, and tagged fat† was injected into the duodenum and the abdomen closed. After 15 minutes the rats were again etherized and the intestines washed with saline, then alcohol and finally with more saline. After a 15-minute absorption period about 30% of the total fat in the mucosa will be tagged fat. The mucosa of 2 rats was freed from intestinal muscle and immediately placed in a boiling water bath for 5 minutes. The intestines of the other animals were placed in beakers of normal saline and allowed to incubate at room temperature for various lengths of time. In the experiment marked with an asterisk (Table I) the intestines were slit lengthwise and placed in oxygenated, glucose Ringer's at 37°C. There was no alcohol used in washing these intestines. In each case when the incubation time was complete the mucosa was removed and immediately placed in a boiling water bath. The mucosal samples were then frozen, dried and extracted. The tagged fat

TABLE I.

Incubation time hr	Increase in total phospholipid tagged fat in dry mucosa %	Phospholipids in dry mucosa %	Increase in phospholipid fatty acids as tagged fat %
0.00	0.00	8.0	0.0
1.75	0.08	3.4	3.3
2.75	—0.04	2.3	1.9
4.50	—0.06	1.1	3.9
6.00*	0.03	1.6	6.7

*This sample was incubated in oxygenated, glucose Ringers at 37°C.
Each figure is from the combined intestinal mucosa of 2 rats.

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[3] Robinson, A., Perlman, I., Rubin, S., and Chaikoff, I. L., *Nature*, 1938, **141**, 119.

[4] Miller, E. S., Barnes, R. H., Kass, J. P., and Burr, G. O., PROC. SOC. EXP. BIOL. AND MED., in press.

† Assistance in the preparation of these materials was furnished by the personnel of Works Progress Administration Official Project No. 665-71-3-69, Subproject No. 6714.

being determined by the method of Miller, *et al.*[4] The fatty acids of the control phospholipids were 1.4% tagged fat. This value was subtracted from that found after incubation and the difference expressed as percent increase in phospholipid fatty acids as tagged fat.

The results in Table I show that on incubation there is an increase in the ratio of tagged fat to untagged fat in the mucosal phospholipids (last column). This would suggest an enzymatic replacement of the phospholipid fatty acids in the incubating tissues. However, there is a steady decrease in the total phospholipids during the incubation period so that the total phospholipid tagged fat (second column) remains constant. The total phospholipid tagged fat is a function of the ratio of tagged fat to untagged fat, and the percent phospholipids in the tissues. A lowering of either of these two factors will result in a decrease in the total tagged fat. In the experiments presented in Table I it is seen that the increase in the ratio of tagged fat to untagged fat is just balanced by the decrease in the percent of phospholipids so that the resulting total phospholipid tagged fat does not significantly change. As yet no explanation of this change is offered, although there appears to be a preferential destruction of the untagged phospholipids.

10795

Effect of Sulfanilamide and Sulfapyridine on Experimental Infections with *Listerella* and *Erysipelothrix* in Mice.

J. R. PORTER AND WILLIAM M. HALE.

From the Department of Bacteriology, State University of Iowa College of Medicine, Iowa City, Iowa.

Within the past few years the literature on the use of sulfanilamide and other related compounds has grown very large. Clinical and experimental results have demonstrated the value of these substances in certain infectious diseases particularly in those infections caused by bacteria of the family *Coccaceæ*. Long and Bliss,[1] Marshall[2] and others have fully reviewed the literature on this subject and no attempt will be made to repeat their work in this short paper.

We[3] noted recently a case of acute meningitis in a small boy resident in the University Hospitals, which was caused by *Listerella monocytogenes.* The chemotherapeutic agent, sulfanilamide, was administered and the patient recovered. We thus became interested in the effect of the drug on the infection. To our knowledge no report has been made on drug therapy for *Listerella* or *Erysipelothrix* infections, especially in experimental animals.

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[1] Long, P. H., Bliss, E. A., and Feinstone, W. H., *J. A. M. A.*, 1939, **12**, 115.

[2] Marshall, E. K., Jr., *Physiol. Rev.*, 1939, **19**, 240.

[3] Wagner, G. W., and Porter, J. R., unpublished data.

The present report deals with the effect of sulfanilamide and sulfapyridine on *Listerella* and *Erysipelothrix* infections in mice. The latter organism was included in our study because Barber[4] reported these two genera and infections to be quite closely related.

The infection of mice with *Listerella* and *Erysipelothrix* produces an acute disease and with a few minor exceptions the symptoms are very similar. We find, as a rule, mice infected with *Listerella monocytogenes* die within 48 to 72 hours, whereas those infected with the same number of *Erysipelothrix* live somewhat longer. The most characteristic post mortem finding in mice infected with *Listerella* is a marked focal necrosis of the liver. Barber[4] observed, and it has been noted by us, that the necrosis is somewhat less marked in mice infected with *Erysipelothrix.* The foci of necrosis are multiple, grayish-yellow in color and scattered throughout the organ. Rarely, if ever, were lesions seen in any of the other organs of animals dead with these diseases.

The organisms used for infecting the animals were cultivated on meat infusion agar slants for 18 to 24 hours and suspended in sterile saline solution to give a nephelometer reading of 15 to 30 million cells per ml. One ml portions were given intraperitoneally to young Swiss mice weighing about 20 to 22 g. Five strains of *Listerella monocytogenes* and 2 strains of *Erysipelothrix* were used in this study.

Sulfanilamide (0.5%) in a 5% glucose-saline solution was administered intraperitoneally in one ml quantities, starting 3 to 4 hours after inoculation with the organisms. The treatment was given approximately every 4 hours thereafter for 5 days. The sulfapyridine was made up as a saturated solution (about 0.1 to 0.2% soluble) and the supernatant administered in 2 ml amounts intraperitoneally. Control animals were kept separate from the treated ones and some of them were injected with a quantity of sterile glucose-saline solution corresponding to that of the treated mice.

The results of this study are presented in Table I and summarized in Table II. The data show that of the total number of mice infected with *Listerella monocytogenes,* 77 out of 80 control animals died,

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[4] Barber, M., *J. Path. and Bact.*, 1939, **48**, 11.

TABLE I.

Effect of Sulfanilamide and Sulfapyridine on Mice Infected with *Listerella* and *Erysipelothrix*.

Organism	No. of organisms given intraperitoneally		No. of mice	Deaths, in days 1	2	3	4	5	6	7	Total Deaths
		Sulfanilamide.									
Listerella monocytogenes "Seastone"	30 million	Treated	10								0
		Controls	10			3	4				7
" " "Ovine"	15 "	Treated	10			1	1		1		3
		Controls	10		2	4	4				10
" " "Murray"	30 "	Treated	10				2				2
		Controls	10		7	2	1				10
" " "Boston"	30 "	Treated	10			2		1			3
		Controls	10		4	6					10
" " "Iowa"	15 "	Treated	20			2	1	1	2		6
		Controls	20		12	8					20
Erysipelothrix No. 32	15 "	Treated	20		8	4	7	1			20
		Controls	20	1	5	6	4	1			17
" No. 33	15 "	Treated	20		7	3	8	1		1	20
		Controls	20		7	4	4				15
		Sulfapyridine.									
Listerella monocytogenes "Iowa"	15 "	Treated	20			1		1			2
		Controls	20		8	12					20
Erysipelothrix No. 32	15 "	Treated	30		10	10	9	1			30
		Controls	30	1	5	13	5	2			26
" No. 33	15 "	Treated	40		18	12	7	1		1	39
		Controls	40		10	20	5				35

TABLE II.
Summary Showing the Effect of Sulfanilamide and Sulfapyridine on *Listerella* and *Erysipelothrix* Infections.

Organism	Treatment	No. of mice	Deaths
Listerella monocytogenes	Sulfanilamide	60	14
	Sulfapyridine	20	2
	Controls	80	77
Erysipelothrix	Sulfanilamide	40	40
	Sulfapyridine	70	69
	Controls	110	93

while 14 out of 60 treated with sulfanilamide died and only 2 out of 20 treated with sulfapyridine died. It can also be seen that most of the control animals succumbed within 3 days whereas the treated animals did not start to die until the third day. When mice were infected with *Erysipelothrix,* treatment with sulfanilamide or sulfapyridine had no beneficial effects, in fact, the treated animals died faster than the controls.

Summary. As is shown in Table II sulfanilamide and sulfapyridine have given good results in treating mice infected with fatal doses of *Listerella monocytogenes.*

The 2 chemotherapeutic agents tested show no helpful effects in the case of *Erysipelothrix* infections. If anything the agents helped to hasten the death of the animals.

From these data it would seem that the mechanism of infection, as influenced by sulfanilamide and sulfapyridine treatment, is different for the two diseases.

10796

An Improved Method for the Study of Intestinal Function.

R. A. BUSSABARGER. (Introduced by O. S. Gibbs.)

From the Department of Pharmacology, University of Tennessee Medical School, Memphis, Tenn.

Thiry[1] described his classical method for the study of intestinal functions. Vella[2] improved this preparation by transforming a blind Thiry pouch into a tube with openings at both ends available for study. From that time many modifications of this basic prin-

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[1] Thiry, L., *Sitzungsberichte d. Akad. Wien, Mathem.-naturw. Kl.I,* 1864, **50**, 77.

[2] Vella, L., *Moleschotts Untersuchungen zur Naturlehre,* 1882, **13**, 40.

ciple have been evolved. In each, however, the segment of gut lies within the peritoneal cavity so it is not possible for the experimenter to observe directly its movements. As such observations are highly desirable, a preparation has been made which differs from the classical in that the isolated segment of gut is taken out of the peritoneal cavity and placed directly under the skin. This allows not only more accurate control of balloon and bolus preparations, but also permits direct visual observations of the behavior of the gut, since movements are most easily seen through the thin overlying skin.

The operation is performed in the following manner. Through a midrectus incision a loop of intestine is selected whose mesenteric vessels are of sufficient length to allow the intestine to be brought freely to the exterior. A segment of 12 to 20 cm is isolated from this loop. The continuity of the remaining gut is restored by "end-to-end" anastomosis and the intestine returned to the peritoneal cavity. The rectus incision is now carefully closed by suturing the peritoneum and fascia around the mesenteric vessels of the remaining isolated segment which lies on the surface of the rectus. By blunt dissection sufficient skin is separated from the underlying fascia until the isolated gut can be housed in the space thus provided. This skin flap is then pulled over the gut and incisions made in it through which each end of the segment is drawn and sutured. Finally, the original skin incision is closed, thus completing the operation. These preparations are less difficult to make and retain in working order than the standard type of Thiry-Vella preparation. However, the same precautions necessary for the maintenance of the Thiry-Vella preparations must be used in these animals.

Using trained dogs we have successfully employed this type of preparation for both bolus and balloon studies with results not only quite equal to the standard type, but with the great advantage, associated with the direct observation of movements, passage of boli, and the position of the recording balloons.

10797

Natural Occurrence of Imperforate Vagina in the Adult Female Albino Rat.*

J. C. Plagge and J. K. Lamar. (Introduced by C. R. Moore.)

From Hull Zoological Laboratory, The University of Chicago.

Occurrence of imperforate vagina with complete absence of the caudal portion has been reported in the adult female albino rat and mouse as a result of prenatal administration of androgens to the mother during pregnancy.[1] The present report deals with a somewhat similar condition discovered in connection with other experiments involving female rats whose mothers had received no treatment during pregnancy.

Imperforate vagina was noted in 9 untreated adult female rats, born of untreated parents between April 15, 1936, and June 16, 1937. Three of the rats were sixth generation descendants of a brother-sister inbred stock; the other 6 cases were from random matings. In each case gross inspection showed no vaginal aperture, and, internally, the lower half of the vagina was missing The upper half of the vagina formed a sac-like structure, closed distally 1 to 6 mm from the perineum, which appeared externally as a continuous, hairless plate between the orifice of the urethra and the anus. The existent portions of the vagina and the uterus were considerably distended with retained fluid in 7 of the animals.

The frequency with which the abnormality was observed in the normal stock colony during a 14-month period was about one in every 150 female rats. The animals were sacrificed for investigation of their internal genital tract at ages ranging from 64 to 442 days.

The most extreme example, case 7, autopsied at 386 days of age, had female reproductive organs which, with their contained fluid, weighed 139 g, almost half of the animal's total body weight of 299 g. The length and width of the vagina were respectively 28x38 mm, the left uterine horn 85x36 mm, and the right uterine horn 56x40 mm. A littermate sister of this rat, case 9, showed

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* This investigation has been aided by a grant from the Rockefeller Foundation to The University of Chicago.

[1] *a.* Greene, R. R., and Ivy, A. C., *Science*, 1937, **86**, 200; *b.* Greene, R. R., Burrill, M. W., and Ivy, A. C., *Science*, 1937, **87**, 396; *c.* Greene, R. R., Burrill, M. W., and Ivy, A. C., Proc. Soc. Exp. Biol. and Med., 1938, **38**, 1 and 4; *d*, Hamilton, J. B., and Gardner, W. U., Proc. Soc. Exp. Biol. and Med., 1937, **37**, 570; *e.* Raynaud, A., *Bull. Biol. de la France et la Belgique*, 1938, **72**, 297.

the same abnormality, although its internal genital organs with fluid weighed 17.3 g compared with a body weight of 189 g. This is the only observed instance of imperforate vagina occurring twice within the same litter.

Seventeen subcutaneous injections of theelin, irregularly administered to case 1 over a period of 34 days before it was sacrificed at the age of 406 days, produced no apparent change in the abnormal vagina.

That the presence of imperforate and bloated vagina does not eliminate the estrous cycle is indicated by case 2 which, by means of an automatically recording revolving cage, was found to run the characteristic 4-day activity cycle for a period of 6 weeks.

Recently,[2] the occurrence of 2 additional cases has come to our attention. These cases occurred in a small colony of hooded rats, of a strain wholly unrelated to ours. A similar anomaly has been described for 3 strains of mice.[3]

These data have been presented as a word of caution in the interpretation of alleged embryonic modifications of the reproductive tract of the female rat foetus as a result of hormone administration to the pregnant mother. The fact that imperforate vaginas were observed in 9 untreated adult female rats, a ratio of about one to every 150 female rats examined, indicates that structural modifications of the reproductive system very similar to those produced experimentally may occur occasionally in a normal rat colony. This possibility would need to be recognized in any evaluation of the effects of hormones on the offspring of treated pregnant rats.

On the other hand, because agreement of experimental results leaves little doubt of the effects of androgens on the secondary sex complex of female rats *in utero,* some hint of explanation of the normal occurrence of a similar condition may be had in the experimental modifications.

Summary. Nine adult female albino rats in a normal stock colony were found with imperforate vagina and absence of its lower half. These data are presented because of the similarity between this condition as observed in untreated rats and that reported by others in female rats whose mothers received injections of androgenic hormones during gestation.

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[2] Beck, N., 1939, personal communication.

[3] Marx, L., *Anat. Rec.*, 1936, **66**, 449.

10798 P

Lactogen Content of Female Guinea Pig Pituitary.

R. P. Reece.* (Introduced by W. C. Russell.)

From the Department of Dairy Husbandry, New Jersey Agricultural Experiment Station.

Previous observations[1] indicated that pituitary glands from lactating guinea pigs contained more lactogen than did glands from sexually mature, non-pregnant, and non-lactating guinea pigs. The intent of this assay work was to compare the lactogen content of pituitary glands from guinea pigs sacrificed in estrum, diestrum, early pregnancy, late pregnancy, and lactation.

Series I. Animals were paired on the basis of body weight, the average body weight of each group being approximately the same. Six guinea pigs were sacrificed in each of the previously mentioned stages.

Series II. Six animals were sacrificed in early pregnancy, late pregnancy, and lactation. However, the average body weights of these groups varied considerably. Lactating animals in both series were sacrificed on the 11th day of the lactation period, the young having been removed 15 hours previously.

The pituitary glands were removed, weighed, and assayed for their lactogen content by injecting the suspended tissue intradermally over the crop glands of common pigeons.

In Series I the pituitary gland of an animal in one group was compared with the gland from an animal of similar body weight in each of the 4 other groups. In Series II the pituitary gland of an animal in one group was compared with the gland from an animal in each of the 2 other groups. In each comparison one-fourth of a pituitary gland was injected over the right crop gland of 3 pigeons and one-fourth of another pituitary gland over the left crop gland of the same 3 pigeons.

In Series I the pituitary glands from guinea pigs in estrum contained slightly more lactogen than did those from animals in diestrum and early pregnancy and slightly less lactogen than did those from guinea pigs in late pregnancy and lactation. Pituitary glands from animals in diestrum contained less lactogen than did those from any of the 4 other goups. In the early pregnancy group the pituitary lactogen content was less than that from animals in estrum, late pregnancy, and lactation and slightly more than that from animals in diestrum. With the exception of animals in lactation, the pituitary lactogen content of the late pregnancy group was more than that of any of the 4 other groups. The pituitary glands from animals in lactation contained more lactogen than did those from animals in any of the 4 other groups.

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* Journal Series Paper of the New Jersey Agricultural Experiment Station, Department of Dairy Husbandry.

[1] Reece, R. P., and Turner, C. W., *Mo. Agr. Exp. Sta. Res. Bul.*, 1937, 266.

TABLE I.
Lactogen Content of Guinea Pig Pituitary Gland.

No. of comparisons	Physiological condition	Avg body wt when sacrificed, g	Avg pituitary wt, mg	Avg No. B.U.* per pituitary gland
Series I				
6	Estrum	564	10.9	16.7
	Diestrum	556	12.4	13.4
6	Estrum			13.6
	Early pregnancy	548†	12.2	12.8
6	Estrum			12.8
	Late pregnancy	553†	12.2	15.2
6	Estrum			13.5
	Lactating	562	13.5	15.5
6	Diestrum			12.8
	Early pregnancy			16.0
6	Diestrum			13.4
	Late pregnancy			14.2
6	Diestrum			14.0
	Lactating			15.5
6	Early pregnancy			13.7
	Late pregnancy			15.3
6	Early pregnancy			11.6
	Lactating			13.9
6	Late pregnancy			12.9
	Lactating			14.7
Series II				
6	Early pregnancy	658†	14.8	12.3
	Late pregnancy	689†	16.2	12.0
6	Early pregnancy			12.2
	Lactating	841	20.9	16.5
6	Late pregnancy			11.2
	Lactating			16.5

{ Assayed in the same group of birds.
* Bird units.
† Body wt minus fetal wt.

In Series II there was no difference in the lactogen content of pituitaries from animals in early pregnancy and late pregnancy. As in Series I, the pituitary glands from animals in lactation contained more lactogen than did those from animals in early and late pregnancy. These results are summarized in Table I.

10799

Plasma pH, CO_2 Content of the Blood and "Tissue Gas" Tensions During Hibernation.

R. T. STORMONT, MARK A. FOSTER AND CARL PFEIFFER. (Introduced by M. H. Seevers.)

From the Departments of Pharmacology, Universities of Wisconsin and Chicago, and the Department of Neurology and Psychiatry, University of Wisconsin.

An increased CO_2 content of the blood as well as a reduction in body temperature occurs in the European marmot during "winter-sleep" according to Dubois,[1] who used these data as support for his carbonic auto-narcosis theory of hibernation. Rasmussen[2, 3] has shown that the CO_2 content of the arterial and venous bloods of woodchucks is increased, but that the CO_2 absorbing power of the blood is decreased during hibernation. The pH of the blood during hibernation, however, has not been determined.

Foster[4] has observed that hibernation can be induced in a large percentage of well fattened and sexually inactive ground squirrels (*Citellus tridecemlineatus,* Mitchill) if, after water is withheld for 48 hours, they are placed in a cold room at 32°-40°F. This procedure was used in these experiments on the same species of ground squirrels. The animals which did not hibernate despite the above procedures are termed "non-hibernators" in this study, although it is possible that they would have hibernated had they been given sufficient time.

Two separate studies have been undertaken. The first has been concerned with the determination of the hematocrit, plasma pH, and CO_2 content of arterial blood by the micro-method of Shock and Hastings.[5] In this study 4 normal, 9 hibernating, and 7 "non-hibernating" animals were used. Blood samples were obtained by heart puncture or decapitation, collected in closed funnels under oil, and immediately drawn into the diluting pipettes. The analyses were then carried out according to the procedure of Shock and Hastings.

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[1] Dubois, R., *Compt. rend. soc. biol.*, 1894, ser. x, i, 821.

[2] Rasmussen, A. T., *Am. J. Physiol.*, 1915, **39**, 20.

[3] *Ibid., Am. J. Physiol.*, 1916, **41**, 162.

[4] Foster, M. A., Foster, R. C., and Meyer, R. K., *Endocrinology*, 1929, **24**, 603.

[5] Shock, N. W., and Hastings, A. B., *J. B. C.*, 1934, **104**, 565.

The results of this study are presented in Table I. The r.b.c. averaged 53.2% in the normals, 57.6% in the hibernating animals, and 58.5% in the "non-hibernating" animals. This increase in the hematocrit is most probably due to dehydration of the animal.

The CO_2 content of the blood averaged 45.5 vol. % in the normal control animals, 71.9 vol. % in the "non-hibernating" animals, and 85.7 vol. % in the hibernating animals.

The plasma pH averaged 7.43 in the normal controls, 7.29 in the "non-hibernators," and 7.10 in the hibernators. The high level of blood CO_2 or a decrease in base bicarbonate might be responsible for the lowering in pH. Rasmussen ascribes the decrease in the absorbing power of the blood for CO_2 during hibernation to a probable decrease in the alkalinity of the blood.

TABLE I.

		Arterial blood		
Animal No.	Hematocrit % cells	pH (plasma) corr. to temp. of animal	CO_2 content Vol. %	
*F 1	53	7.52	40.0	Normal-stock animal
F 2	52	7.38		” ” ”
M 3	55	7.45	45.3	” ” ”
M 4	54	7.38	51.2	” ” ”
Avg	53.5	7.43	45.5	
F 30	65	7.30	67.7	Non-hibernator (in cold for 5 days without H_2O)
F 28	55	7.35	71.3	” ”
F 32	59	7.25	76.9	” ”
M 19	60	7.27		” ”
M 13	59	7.25		” ”
M 15	58	7.40		” ”
M 14	54	7.20		” ”
Avg	58.5	7.29	71.9	
M 10	57	7.20	89.7	Hibernator
M 11	64	7.11	79.5	”
M 12	60	7.07	77.7	”
F 26	57	7.01	89.2	”
M 27	53	7.02	73.8	”
F 25	55	7.19	93.4	”
F 32	55	7.04	92.8	”
M 21	56	7.04	88.0	”
M 20	62	7.19	87.7	”
Avg	57.6	7.10	85.7	
M 37	67	7.27	57.0	24 hr out of hibernation

*F—female. M—male.

In the second study "tissue gas depots" were established in the peritoneal cavities of 5 normal ground squirrels according to the procedure developed by Campbell.[6] Gas samples were drawn from these pockets without air contamination and analyzed for CO_2 and O_2, using the micro-gas analysis apparatus described by Seevers and Stormont.[7] From these data the CO_2 and O_2 gas tensions in mm of Hg were calculated* (corrected to 740 mm bar. press.). Samples were taken approximately every second day before, during, and after hibernation.

Fig. 1 shows the changes obtaining in the "peritoneal tissue gas tensions" before and during hibernation. The final readings were made when the animals had been removed from the cold-room and given food and water. Lowered metabolism probably accounts for

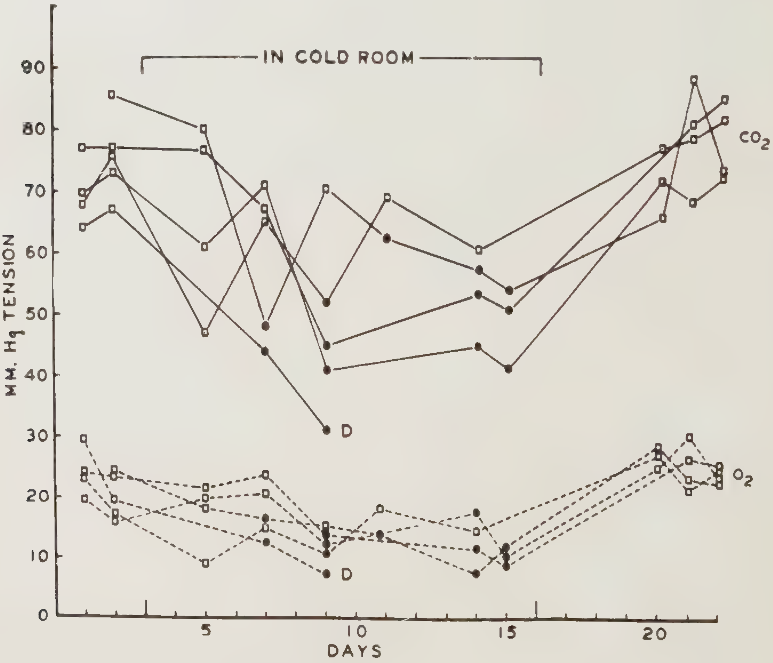

FIG. 1.

CO_2 tensions and O_2 tensions in the peritoneal cavities of five animals. White squares—awake; black circles—hibernating; D—animal found dead next day.

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[6] Campbell, J. A., *J. Physiol.*, 1923, **57**, 273.

[7] Seevers, M. H., and Stormont, R. T., *Ind. and Eng. Chem.* (Anal. Ed.), 1937, **9**, 39.

$$\text{*mm tension} = \text{Vol. \% dry gas} \times \frac{\text{Obs. Bar. Press.} - Q}{100} \times \frac{740}{\text{Obs. Bar. Press.}}$$

Q = tension of H_2O vapor at temp. of animal.

the decrease in both the CO_2 and O_2 tensions. There is a definite small mortality among ground squirrels hibernating for the first time. It is, hence, of interest to note the low tensions of O_2 and CO_2 in one animal (D, Fig. 1) 24 hours before death from hibernation.

The equation developed by Hastings and Shock[8] for the calculation of the arterial blood CO_2 tension (pCO_2) from the hematocrit, plasma pH, and CO_2 content values was modified to apply theoretically for blood at 4°C, and an attempt was made to calculate the CO_2 tensions of the bloods of the hibernating animals. The resulting values averaged 50% higher than the CO_2 tensions obtained by direct tonometry in the peritoneal cavity. The validity of the use of the Hastings-Shock equation extrapolated to 4°C may be questioned on the bases that the arterial O_2 saturation was not determined, the state of the CO_2 in the blood under these conditions is not definitely known, and the ionic or protein concentrations in the blood of these dormant animals may be significantly different from that in man or the ox. Roughton[9] suggests that the carbamino mechanism might play a more important rôle in CO_2 transport in the case of cold blooded animals since carbamino compounds are more readily formed at lower temperatures.

It should be noted that the rise in arterial CO_2 content and the fall in the pH of the blood are similar to those noted in asphyxia.[10] The hibernating animal is suggested as a possible test object for physiological and pharmacological studies of animals in a fixed acidotic state.

Summary. During hibernation arterial blood CO_2 content increases to an average of 85.7 vol. % compared to an average normal of 45.5 vol. %. The plasma pH changes from an average of 7.43 to 7.10. The tensions of CO_2 and O_2 in the peritoneal gas pocket decrease significantly.

The authors wish to thank Drs. A. B. Hastings and N. W. Shock for helpful advice during this study.

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[8] Hastings, A. B., and Shock, N. W., *J. B. C.*, 1934, **104**, 575.

[9] Roughton, F. J. W., *Physiol. Rev.*, 1935, **15**, 241.

[10] Koehler, A. E., Behneman, H. M. F., Berrell, O. E., and Loevenhart, A. S., *Am. J. Physiol.*, 1925, **74**, 590.

10800

Study on the Photo-Oxidation of Blood Plasma and Tissues in Buckwheat Disease.

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The effect of irradiation of animals fed on buckwheat is well known and has been described in detail by several authors.[1-6] The majority of the workers in this field assume that buckwheat disease is due to a substance contained in this plant which after ingestion renders the animals susceptible to light within the visible range of the spectrum.[1-5]

Since photo-dynamic action is characterized by the oxidation of an acceptor in presence of a sensitizer during irradiation, studies were made on the oxygen consumption of irradiated blood plasma and tissues of animals fed on buckwheat as well as of controls. It was thought that if a photodynamic substance were present, there should be a greater oxygen consumption during irradiation of plasma and tissues of sensitized animals than during irradiation of similar substrates of controls.

Materials and Technic. Thirty-six guinea pigs, weighing from 600 to 800 g were fed exclusively on fresh buckwheat plants and seeds from 2 weeks to 3 months. The animals were kept in cages covered with black cloth. Thirty-three control guinea pigs were fed on stock diet and also kept in the dark. All animals seemed entirely well as long as they were kept in the dark; they gained weight and some females had litters during this period; the young appeared normal. In order to prove that animals were sensitive to light, albino animals fed on buckwheat and controls were exposed to sunlight in glass jars covered with glass tops. It was found that guinea pigs exhibited typical symptoms of photo-sensitivity after 4 to 6 days of feeding on buckwheat. Heart punctures of sensitive animals and controls were made from time to time and the blood plasma or serum was examined through the spectroscope or the spectropho-

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[1] Oehmke, W., *Zentralbl. f. Physiol.*, 1909, **22**, 685.

[2] Meridan, L., *Arch. f. Anat. u. Physiol.*, 1915, **39**, 161.

[3] Sheard, C. H., Caylor, H. D., and Schlotthauer, C., *J. Exp. Med.*, 1928, **47**, 1013.

[4] Hausmann, W., and Zaribnicky, *Klin. Wchnschr.*, 1929, **8**, 74.

[5] Hausmann and Haxthausen, *Strahlentherapie*, 1929, Sonderband XI.

[6] Lutz, H. E. W., and Schmid, G., *Biochem. Z.*, 1930, **226**, 67.

tometer. Samples of plasma or serum were placed in respirometers and the oxygen consumption before, during and after exposure to light was studied in the water bath at 37.5°C. The details of the apparatus, temperature control and light source are given in a previous communication.[7] Experimental animals and controls were sacrificed at intervals and certain organs were finely sliced with a sharp razor blade. After weighing, tissue slices were placed in respirometers containing 1 cc of Ringer's solution of pH 7 and the oxygen consumption was studied before, during and after exposure to light in the water bath. Routine autopsies were performed on all animals.

Results. Spectroscopic and spectrophotometric examinations of plasma or serum of guinea pigs fed on buckwheat from 2 weeks to over 3 months never showed any abnormal absorption bands; traces of hemoglobin were frequently encountered.

The results of the studies on the oxygen consumption during irradiation of tissue slices and plasma of animals fed on buckwheat as well as of controls are presented in Table I. The figures give the average of cmm of oxygen consumed during 10 minutes of a 30-minute period. Each figure represents the mean of from 9 to 30 observations or an average of 17 experiments. The column showing the difference between the readings divided by the difference of the probable errors always pertains to the figures before and during irradiation.

It can be seen that there is no significant difference between the oxygen consumption of organs of animals fed on buckwheat during irradiation and controls. There is the usual increase in oxygen consumption of plasma during irradiation, which occurs in both experimental animals and controls.

Autopsies of guinea pigs fed on buckwheat never showed any characteristic lesions. The gall bladder of the experimental animals was usually larger and contained more bile than that of controls which was probably due to the feeding of green plants. Spectrophotometric studies of the bile of the gall bladder of animals fed on buckwheat never showed any absorption bands different from those seen in the bile of controls.

In the absence of any demonstrable difference in the oxygen consumption of plasma and tissues between animals fed on buckwheat and controls, the question arises whether buckwheat disease is due to the presence of a photodynamic substance. The behavior during irradiation of guinea pigs fed on buckwheat is, however, identical to that of animals effectively sensitized by the parenteral injection of a photosensitizing substance, for instance, hematoporphyrin.

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[7] Smetana, H., *J. Biol. Chem.*, 1938, **124**, 667.

TABLE I.

Oxygen Consumption of Blood Plasma and Tissues of Guinea Pigs Fed on Buckwheat and Controls. Average of cmm of O_2 consumed per mg of tissue during 10 min of 30 min period.

	Exposure to Light (400μ Amps.)							
	Experimental Animals				Controls			
				Diff.				Diff.
Organs	Before	During	After	PE Diff.	Before	During	After	PE Diff.
Skin	1.08±.04	1.12±.05	.84±.06	.62	1.3±.08	1.6±.11	1.3±.11	2 1
Subcut. tissue	.73±.04	.79±.1	.67±.03	.06	.7±.04	.7±.03	.6±.06	0
Liver	13.2±.18	13.6±.14	13.1±.16	1.7	12.2±.16	12.3±.16	11.7±.12	.043
Spleen	11.0±.12	11.1±.14	12.8±.21	.05	12.6±.34	12.4±.49	10.7±.92	0
Kidney	27.6±.27	27.7±.33	26.5±.21	.03	28.2±.28	26.5±.34	26.9±.32	0
Brain	7.9±.21	7.0±.29	6.5±.54	0	7.8±.45	7.0±.52	5.6±.28	0
Blood Plasma	.4±.05	1.86±.13	.4±.09	9.73	.4±.062	1.85±.15	.4±.1	8.95

Since symptoms in buckwheat disease are very pronounced and start almost immediately upon exposure of animals to sunlight one would expect a measurable concentration of a photosensitizing substance in the blood as well as in some of the tissues, if this substance were the cause of this disease. If such a substance does exist, its concentration in the blood plasma and in the tissues is too low as to effect the oxygen consumption during irradiation *in vitro*. Compared with the effect of concentrations of hematoporphyrin on plasma and tissues this would be the case in the neighborhood of concentration 10^{-6}; such a low concentration of sensitizer on the other hand would hardly produce the marked actual effect *in vivo*.[7, 8] Since all experiments *in vitro* were carried out with the exclusion of ultraviolet and infrared rays the question arises whether the effective rays, causing symptoms of buckwheat disease, lie in the invisible part of the spectrum. This was quickly disproved by a few experiments where irradiation of plasma and tissues of sensitive animals was carried out in quartz containers, using a light source rich in infrared and ultraviolet rays, which was projected on the respirometers through a quartz window. The results were in no way different from those obtained when identical material was irradiated in glass respirometers. Besides, effective irradiation of sensitive animals was carried out in glass jars covered with glass tops. Moreover, most workers in this field[2–6] have found that the invisible parts of the spectrum were ineffective in producing symptoms of buckwheat disease.

Although many of the authors[1–5] suggest the presence of a photodynamic substance in buckwheat disease, no such substance has as yet been conclusively demonstrated. The suggestion that phylloporphyrin or cholehematin may be the photodynamic substances causing buckwheat disease[3] was based on absorption bands found in a single sample of swine serum. It has been ruled out by several authors[3, 6] that ordinary chlorophyl or a specific chlorophyl of buckwheat was the photodynamic substance causing the disease. Lutz and Schmid[6] left the question open whether buckwheat disease is due to a photosensitizing substance or to intoxication.

Because of the failure to demonstrate any photodynamic substance responsible for the sensitivity to light of animals fed on buckwheat, it might be suggested that feeding of buckwheat may possibly cause a change in the tegument of the animal, rendering the skin more susceptible to irradiation, similar to the effect of sunlight in relation to skin lesions in pellagra. Payne and Perlzweig[9] have shown that there

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[8] Smetana, H., *J. Biol. Chem.*, 1938, **125**, 741.

[9] Payne, S. A., and Perlzweig, *J. Clin. Invest.*, 1935, **12**, 899.

exists an abnormal sulphur metabolism in pellagra which is reflected in the susceptibility of the skin to irradiation and is specifically related to the dermatitis of this disease. According to these authors the relatively high cystine content in wool and hair is of physiological importance in the protection of the organism against harmful effects of prolonged exposure to light. In this respect the changes and the falling out of hair of animals fed on buckwheat, as reported by Oehmke,[1] are of interest and perhaps of significance.

Conclusions. 1. There is no abnormal increase of the oxygen consumption during irradiation of plasma and tissues of various organs of guinea pigs made susceptible to light after prolonged feeding with buckwheat. 2. There is no conclusive evidence that the susceptibility to light of animals in buckwheat disease is due to a photodynamic substance.

10801

Cause of Increased Fragility of Erythrocytes in Congestive Heart Failure.

JOHN WALLER. (Introduced by M. D. Altschule.)

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Increased fragility of the red blood cells has been noted in certain patients with severe congestive heart failure.[1, 2] With recovery from heart failure the minimum osmotic resistance of the red cells to hypotonic salt solutions returns to normal.[2, 3] It was of interest to study the effect of anoxemia and stasis on blood in the capillaries and in the veins in producing these changes.

The studies were made on 10 convalescent ward subjects with no demonstrable cardiac, respiratory or hematologic disease. Using the hypotonic saline method of Daland and Worthley,[4] the minimum osmotic resistance of the red blood cells obtained from the finger tips was observed. Complete circulatory arrest was produced by means of a standard blood pressure cuff applied to the right upper arm and rapidly inflated to a level 50 mm of mercury above the systolic pressure; this pressure was maintained for 10 minutes. Control observations were made on blood drawn from the finger tips of the left hand in each instance. Each finger tip, after lancing, was immersed in physiological saline containing a few drops of potassium and ammonium oxalate mixture as anticoagulant. In experiments 1, 2, and 3 all of the solutions were first boiled and cooled and layered with mineral oil and the entire procedure was thus carried out anaerobically. In all the remaining experiments, on the other hand, no attempt to prevent gaseous exchange with the atmosphere was made.

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[1] Franceson, M., *Minerva Med.*, 1936, **37**, 255.

[2] Waller, J. V., and Blumgart, H. L., to be published.

[3] Butler, G. G., *Quart. J. Med.*, 1912-13, **6**, 145.

[4] Daland, G. A., and Worthley, K., *J. Lab. and Clin. Med.*, 1935, **20**, 1122.

Various technics were employed in an endeavor to investigate the fragility of venous blood after stasis. In experiments 1, 2, and 3 ten cc of blood from a cubital vein was removed anaerobically before the release of the tourniquet. In 2 additional subjects (9 and 10), after 15 minutes of complete circulatory arrest, blood was withdrawn from a cubital vein in successive 15 cc samples as the forearm was wrapped, progressing from hand to elbow, in an Esmarch bandage. In this fashion from 60 to 90 cc of blood was withdrawn. The fragility of a sample from each syringe was observed and compared with that of venous blood taken from the other arm without stasis and with the fragility of finger-tip bloods taken before and after stasis.

Results. The decrease of the resistance to lysis in hypotonic saline of the red blood cells obtained from the finger tips was noted in 7 of 10 instances (Table I). Bloods 4 to 10 were thoroughly mixed with air while bloods 1, 2, and 3 were handled anaerobically.

In experiments 1, 2, and 3 the initial 10 cc of venous blood, even

TABLE I.
Beginning Fragility of Erythrocytes Obtained from Blood of Normal Finger or Arm Compared to That Obtained from Arm in which Stasis Had Been Produced.

	Blood from finger tips		Blood from cubital vein	
Subject Number	Without Stasis	With Stasis	Without Stasis	With Stasis
1	.44% NaCl	.48	.44	.44
2	.44	.50	.44	.44
3	.44	.48	.44	.44
4	.44	.48		
5	.48	.50		
6	.46	.46		
7	.44	.46		
8	.48	.48		
9	.44	.48	.44	$.44^{1}$,* $.46^{2}$, $.48^{5}$, $.46^{6}$
10	.46	.46	.48	$.48^{1}$, $.52^{2}$, $.52^{3}$, $.50^{5}$

* Small numbers refer to the sequence of withdrawal of the samples.

though handled anaerobically failed to show any change in fragility. Successive samples of venous blood from subjects 9 and 10, however, show a definite alteration of fragility (Table I).

The association of increased fragility of the red blood cells with the more severe degrees of cyanosis in congestive heart failure suggests that anoxia may account for the alteration of the fragility.

Previous workers[3, 5, 6] found that venous blood was more fragile than either arterial or capillary blood. The fragility of these bloods could be reduced to that of the arterial blood by bubbling oxygen through the sample of whole blood before clotting occurred. They also demonstrated the marked increase in fragility which occurs when carbon dioxide is bubbled through whole blood. This could also be reversed by subsequently bubbling oxygen through the blood. In the present study each sample of blood was dark and "cyanotic" on withdrawal from finger or vein. After the saline-cell suspension had been exposed to air by repeated inversion in tubes half filled with air, the red cells all took on the bright red color of oxyhemoglobin. Since definite alterations of the fragility of the red cells were present after equilibration with room air (subjects 4, 5, 7, 9, and 10) it is apparent that neither low oxygen tension nor a high tension of carbon dioxide was responsible for these changes.

The finding of increased fragility, after stasis, of blood obtained by capillary puncture or by venipuncture after expressing the capillary blood into the veins, in contrast to the absence of such a finding in blood which had merely been stagnant in the veins, suggests that the increased fragility results from certain effects of tissue metabolism under the conditions of the experiments.

Von Stejksal[7] observed minimal increase of fragility in 2 of 5 patients after the production of stasis in one arm previously rendered "anemic" by elevation. He thought that the change in osmotic resistance of the red blood cells might be due to the action of a lysin present primarily in serum from anemic blood. Since the subjects of the present investigation were not anemic, von Stejksal's hypothesis is not tenable.

Conclusions. Increase in fragility of the red blood cells to hypotonic saline occurs in capillary blood in the presence of circulatory arrest. This is similar in degree and direction to that observed in congestive heart failure. These alterations may also be observed in certain samples obtained from a vein which contains an appreciable amount of capillary blood.

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[5] Dacie, J. F., and Vaughan, J. M., *J. Path. and Bact.*, 1938, **46**, 341.

[6] Creed, E. F., *J. Path. and Bact.*, 1938, **46**, 331.

[7] v. Stejksal, K. R., *Wien Klin. Wchnschr.*, 1909, **1**, 661.

10802 P

Failure of an Acute Inflammatory Process to Extend into Area Previously Injected with India Ink.

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The observations of Menkin[1] and of Burrows[2] on the failure of the diffusion of trypan blue into areas of inflammation when the dye is injected intradermally near the inflamed site, are interpreted upon the basis of thrombotic occlusions in the lymphatics and on the interference produced by a fibrinous exudate. It seemed of interest then, to study the mechanism of the spread of an acute inflammatory process into the site of a chronic inflammatory reaction. This has been made possible by the intradermal injection of India ink and at subsequent times, by the injection of pneumococci into the adjacent skin.

Preliminary intradermal injections of 0.4 cc of India ink in saline dilutions from 1 :4 to 1 :50 were made on the sides of white rabbits. Subsequently either .002 cc of Type I or 0.1 cc of Type III pneumococcus 18-hr. broth cultures were injected intradermally 1 to 2 cm above this site. These inoculations were made at intervals from 24 hours to 11 weeks after the ink was injected. Inflammation was also induced in other rabbits by the intradermal injection of 0.2 cc of oil of citronella. This oil produces a more marked induration and a more slowly spreading inflammation than that produced by the pneumococci. A description of the inflammation produced in the skin of rabbits by pneumococci has been given by Rhoads and Goodner.[3] The animals were killed at intervals and sections of the skin were made.

There is present after 24 hours slight hyperemia, edema and leucocytic infiltration in the area of skin injected with India ink. This reaction slowly subsides and after a month there is essentially no cellular reaction at the site of the ink injection. From the site of the inoculation of the pneumococci there develops diffuse edema and hyperemia and rapid spread of the infection downward. The direction of spread is influenced by the lymphatic drainage and by gravity. When the bacteria are injected 2 or 3 cm above the area of India ink the edema and hyperemia pass around but do not pass

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[1] Menkin, V., *J. Exp. Med.*, 1931, **53**, 171.

[2] Burrows, H., *Localization of Disease*, Wm. Wood and Co., N. Y., 1932.

[3] Rhoads, C. P., and Goodner, K., *J. Exp. Med.*, 1931, **54**, 41.

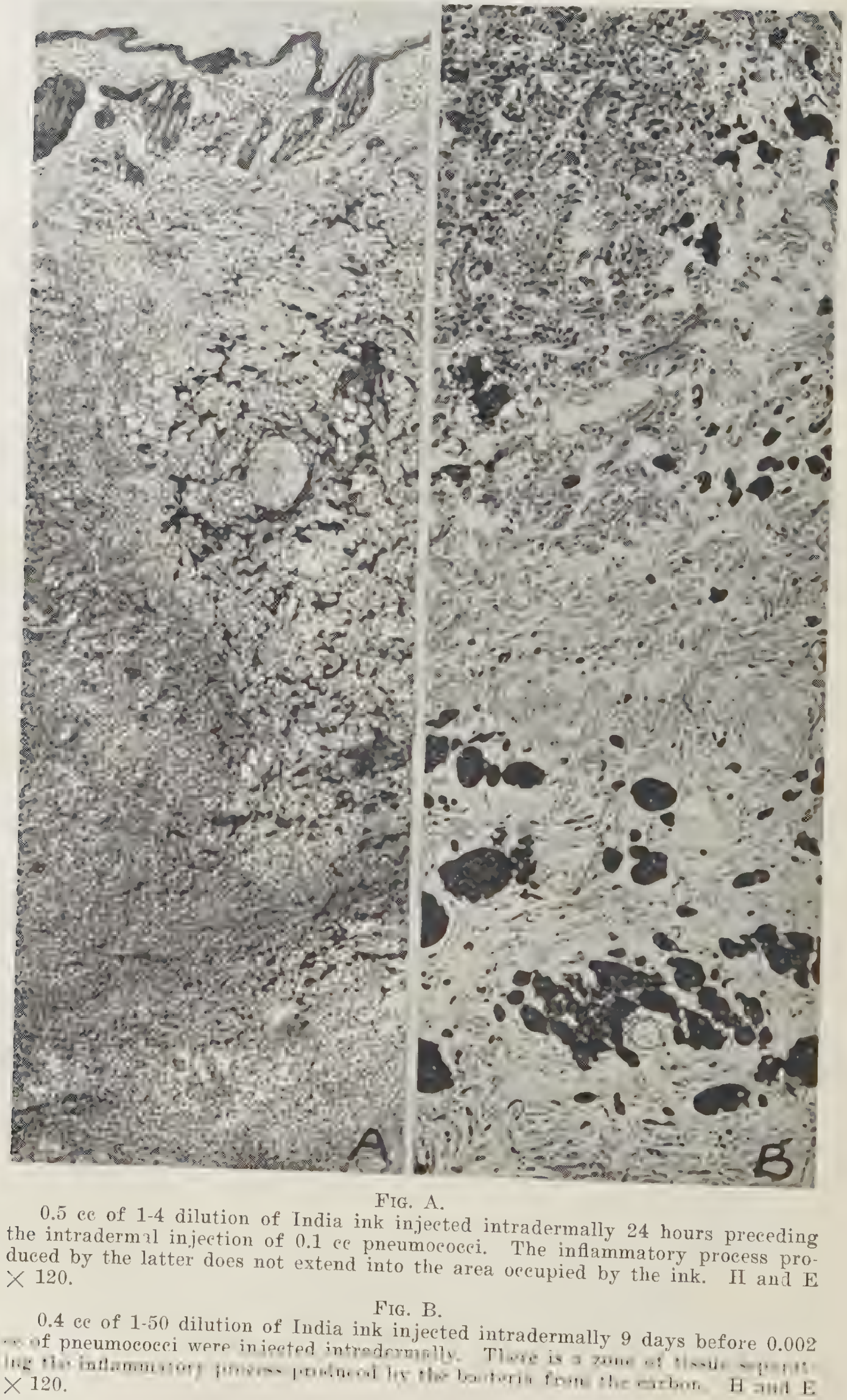

FIG. A.

0.5 cc of 1-4 dilution of India ink injected intradermally 24 hours preceding the intradermal injection of 0.1 cc pneumococci. The inflammatory process produced by the latter does not extend into the area occupied by the ink. H and E × 120.

FIG. B.

0.4 cc of 1-50 dilution of India ink injected intradermally 9 days before 0.002 cc of pneumococci were injected intradermally. There is a zone of tissue separating the inflammatory process produced by the bacteria from the carbon. H and E × 120.

through the pigmented area. (Fig. A.) When the pneumococci are injected 24 hours following the injection of India ink, the area is not so refractory as it is after a period of 11 weeks. At this time there is an area around the site of injection of the ink that is not invaded by leucocytes. (Fig. B.) Similarly the more slowly spreading inflammation induced by the oil injections also did not extend into the ink areas.

Immediately and for several days following the injection of the ink the carbon particles are dispersed throughout the tissue. There are collections of polymorphonuclear leucocytes around the periphery of the blood vessels in these pigmented areas but apparently these leucocytes are not able to wander through the tissue in such areas as readily as they do in the normal skin. There is an increase in the amount of connective tissue around these areas of carbon. A further study of these sections shows that the ink is located in the tissue spaces as well as in the lymphatics.

It is interesting to note that a mild, chronic reaction, such as that produced by the intradermal injection of sterile ink tends to prevent the inward diffusion of fluids, just as Menkin has observed the failure of trypan blue to diffuse into areas of acute inflammation.

If, however, the pneumococci are injected directly into the ink areas, then the inflammation spreads from these sites. This is probably due in part to the disruption caused by the pressure of the subsequent injection.

It is evident from these experiments that skin injected with India ink does not permit the infiltration of bacteria, edema fluid, and leucocytes from an advancing inflammation. The degree of limitation of the spread of the inflammation progresses with the period of time that elapses between the injections of the India ink and the irritant. This process appears to be influenced by scarring, by the plugging of the lymphatics with carbon particles and also by the accumulation of carbon in the tissue spaces. Similar results were obtained when trypan blue in dilutions from 1:50 to 1:500 was substituted for India ink. The change in the consistence of the tissue seems also to contribute to the difficulty of the extension of the bacteria, the infiltration of the tissues by edema fluid and the migration of polymorphonuclear leucocytes into the dye areas.

10803

Relation Between Amphibian Water Retention Principle and Oxytocic Activity of Pituitary Extracts.

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Heller,[1] Steggerda and Essex,[2] Oldham,[3] and Boyd and Brown[4] have found that the injection of oxytocic fractions of posterior pituitary extracts (such as pitocin) cause frogs immersed in water to take up more water than injection of pressor fractions (such as pitressin). These results suggest that the water uptake in frogs may be related more to the oxytocic principle than to the pressor principle. If such were so, it should be possible to demonstrate a quantitative relationship between the amount of frog water uptake principle and oxytocic principle in extracts of the neurohypophysis. Such a relationship was found to exist but determination of the nicety of the correlation was limited by the variations inherent in the methods of assay. The finding of this correlation suggests either that these two principles are the same substance or that they separate proportionately in the same methods of extraction.

The amount of the frog water uptake principle was estimated on leopard frogs *(Rana pipiens)* by a method recently devised in this laboratory.[5] The method of assay consisted in injecting into the dorsal lymph sac, 5 different increasing doses of extract per 10 g body weight of frog. Each dose was injected into 10 frogs acclimatized to water at 4°C and the dosage range was judged from preliminary assays with one or 2 frogs to a dose. The frogs were carefully dried and weighed before injection and again at 18 hours after injection at which time previous results had shown that they have taken up their maximum load of water. This peak was held for several hours when the assay was performed at the low temperature of 4°C, which was the advantage of using this low temperature, as it permitted 2 weighing assays and consequently permitted a greater number of animals being used at one time.

The mean percentage uptake of water in each group of frogs was then found and these means were plotted against dosage. To be acceptable, a curve with 3 points along the maximal plateau was required; if such a curve was not obtained, a more suitable series of doses was selected and the assay repeated. From this curve we selected our unit dose which we have defined[5] as the smallest amount of extract, per 10 g body weight of leopard frogs in water equilibrium at 4°C, required to produce in 18 hours in 10 frogs a mean gain in weight which is within 25% of the greatest mean percentage gain in weight of at least 3 similar groups of frogs yielding distributed, maximal plateau gains. The figure of 25% was semi-arbitrary and was based upon a statistical analysis of the expected variation of maximal responses discussed in our previous communication.[5]

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[1] Heller, J., *Arch. exp. Path. u. Pharmakol.*, 1930, **157**, 323.

[2] Steggerda, F. R., and Essex, H. E., PROC. SOC. EXP. BIOL. AND MED., 1934. **32**, 425.

[3] Oldham, F. K., *Am. J. Physiol.*, 1936, **115**, 275.

[4] Boyd, E. M., and Brown, G. M., *Am. J. Physiol.*, 1938, **122**, 191.

[5] Boyd, E. M., and Mack, E. G., *Endocrinology*, in press.

To determine the variation one may expect to get with this method, 12 repeated assays, with a total of at least 50 frogs to an assay, were performed on the same sample of pituitrin. The mean number of mm^3 of this pituitrin found to contain one frog unit was 5.7 with a standard deviation of 1.9, giving 33 as the value for Pearson's coefficient of variation.

We have named this principle, assayed as above, the amphibian water retention principle.[5] We have qualified the title with the word 'amphibian' because the exact relation between this principle and the principle which inhibits water diuresis in mammals is not yet established. We have used the descriptive title 'water retention' rather than 'water uptake' because Boyd and Whyte[6, 7] have presented evidence which indicates that the uptake of water is probably due to an inhibition of the normal loss of water with little or no effect on water intake. This conception of the mechanism of the pituitrin-uptake reaction has been substantiated by further studies in this laboratory.[8]

Twelve different batches of Solution of Posterior Pituitary, U.S.P. XI, or of Pituitary (Posterior Lobe) Extract, B.P. 1932, obtained from 4 different manufacturers were assayed for amphibian water retention principle. The units per ml were then compared with the international oxytocic units per ml and this comparison is presented in Table I. Since the extracts used were pharmacopoeial products assayed by standard methods and produced by reputable manufacturers, we accepted the assay of the manufacturer for the international oxytocic units at the time that the extract was put upon

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[6] Boyd, E. M., and Whyte, D. W., *Am. J. Physiol.*, 1938, **124**, 759.

[7] Boyd, E. M., and Whyte, D. W., *Am. J. Physiol.*, 1939, **125**, 415.

[8] Boyd, E. M., Clark, K. J., and Smith, A. E., unpublished data.

TABLE I.
Water Retention and Oxytocic Units of Posterior Pituitary Extracts.

Manufacturer	Year of Manufacture	Water Retention Units/ml	Oxytocic Units per ml	Water Retention Units / Oxytocic Units
Parke, Davis: Obstetrical	1934	40	10	4
” ” Surgical	1933	200	20	10
” ” ”	1938	100	20	5
” ” ”	1937	200	20	10
” ” Pitocin	1938	200	20	10
” ” Pitressin	1938	<10	<1	10
Connaught	1937	100	10	10
Frosst	1932	100	10	10
”	1933	100	10	10
”	1938	50	10	5
Squibb: Surgical	1937	100	20	5
” Obstetrical	1937	100	10	10

the market. At the present time we are not prepared in this laboratory to reliably assay for international oxytocic units. We were reasonably certain that the manufacturers' assays were carefully done. This was checked by correspondence and in 2 instances by a personal visit by one of us to the control laboratory in which the extracts were assayed. In the case of each product it appeared that at least 4 comparisons with standard had been made and that Pearson's coefficient of variation of the results would probably average about the same as that found in our assay for amphibian water retention principle.

With the variations inherent in both methods of assay so great, it was obvious that any correlation found could be only approximate at the best. Most of the pituitary extracts were diluted to contain either 10 or 20 international oxytocic units per ml and if a correlation existed, one would expect to find some factor times these numbers, of amphibian water retention units per ml. This factor is given in the 5th column of Table I and is derived from the figures given in columns 3 and 4.

There were found from 4 to 10 times as many amphibian water retention units per ml as international oxytocic units. In 8 of the 12 extracts there were 10 times as many amphibian water retention units as oxytocic units. Considering the variation inherent in both methods of assay, this appeared reasonably significant.

Hence the correlation coefficient of figures in columns 3 and 4 of Table I was calculated after the formulae of Wylie.[9] The correlation coefficient was found to be +0.8 with a probable error of 0.07. The coefficient being positive and near 1, the correlation between the units per ml was positive and significant. The coefficient was over 11 times its probable error so that the odds were overwhelming that it was significant.

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[9] Wylie, J., Ch. XIV of Currie, J. R., *A Textbook of Hygiene*, Livingstone, Edinburgh, 1930.

These results indicate that there is a correlation between the content of international oxytocic units and amphibian water retention units per ml of posterior pituitary extracts. The methods of assay were not sufficiently accurate to show just how close this correlation may be. On the other hand, the water diuresis inhibiting principle in mammals is usually considered more closely related to the pressor principle.[10, 11] With the evidence available, this would appear to be a characteristic difference between the 2 principles. It is possible, however, that the difference in the methods of assay may account for this apparently different distribution and this is now being investigated in this laboratory.

It will be noted in Table I that there were little or no differences in the content of amphibian water retention units per ml of old versus recent extracts. We are not in a position to know if the content of oxytocic units per ml in the same extracts was also unaffected, or seemingly unaffected, by age. The evidence suggests, however, that the amphibian water retention principle is stable in these extracts for periods of up to 7 years at least.

Summary. Twelve posterior pituitary extracts were found to contain 4 to 10 times as many amphibian water retention units per ml as international oxytocic units. Variation in the technics of assay prevented an estimation of the nicety of this correlation.

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[10] Hemingway, A., and Peterson, J. M., *J. Physiol.*, 1929, **67**, 24.

[11] Gargle, S. L., Gilligan, D. R., and Blumgart, H. M., *New Eng. J. Med.*, 1928, **198**, 169.

10804

Effect of Chlorophyll Derivatives and Related Compounds on the Growth of *M. tuberculosis.**

Sylvester Daly, George Heller, and Erich Schneider. (Introduced by C. W. Jungeblut.)

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Attenuation in the virulence of the bovine tubercle bacillus by cultivation on ox-bile media (Calmette and Guérin,[1] Bouquet and Nègre[2]) suggests the evaluation of the individual constituents of bile as to their possible effect on *M. tuberculosis.* However, of the many bile constituents, only certain bile acids and their salts have been studied in detail regarding their action on the tubercle bacillus (Proskauer and Beck,[3] Deyke and Much[4]). Present in bile are certain decomposition products of chlorophyll, particularly phylloerythrin (Loebish and Fischler[5]) and pyrroporphyrin (Rothemund[6]). It was therefore considered desirable to determine whether certain water soluble chlorophyll derivatives and related porphyrins affected the growth of these organisms.

The chlorophyll compounds, selected because of their solubility in water, were chlorin-e, the copper complex of chlorin-e, and chlorophyllin-a. Besides these compounds, closely related to chlorophyll, certain porphyrins were studied—pyrroporphyrin, representing the chlorophyll porphyrins and deuteroporphyrin, representing the blood porphyrins.

Chlorophyllin-a was prepared following Willstaetter's[7] method, by alkaline cleavage of chlorophyll-a. It is a tri-carboxylic acid in which two of the carboxyl groups occur as in the original chlorophyll molecule, while the third one is formed by the splitting of the isocyclic ring. To make chlorophyllin water soluble, the tri-sodium salt was prepared.

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* This work was supported by the Jovan Laboratories, Inc., Gift Fund.

[1] Calmette, A., and Guérin, C., *Ann. d. l'Inst. Past.*, 1908, **22**, 689.

[2] Bouquet, A., and Nègre, L., *Ann. d. l'Inst. Past.*, 1921, **35**, 142.

[3] Proskauer, B., and Beck, M., *Z. f. Hyg.*, 1894, **18**, 128.

[4] Deyke, G., and Much, H., *Münch. Med. Woch.*, 1909, **56**, 1985.

[5] Loebisch, W. F., and Fischler, M., *Monatsch. f. Chemie*, 1903, **24**, 335.

[6] Rothemund, P., *J. Am. Chem. Soc.*, 1935, **57**, 2180.

[7] Willstaetter, R. M., *Untersuchungen Ueber Chlorophyll*, J. Springer, Berlin, 1913.

Chlorin-e is prepared by splitting off the magnesium atom from chlorophyllin by acid treatment. However, it is more convenient to prepare it from pheophytin-a, a compound which corresponds to chlorophyll, but is magnesium-free. Alkaline cleavage of pheophytin-a, following Willstaetter's process, yields chlorin-e in the same way as chlorophyll, with alkaline treatment, yields chlorophyllin. Here again the trisodium salt of chlorin-e was prepared to make the compound water-soluble.

$$\begin{tikzcd}
\text{chlorophyll-a} \arrow[r, "\text{Acid treatment}"] \arrow[d, "\text{alkaline cleavage}"] & \text{pheophytin-a} \arrow[d, "\text{alkaline cleavage}"'] \\
\text{chlorophyllin-a} \arrow[r, "\textbf{Acid treatment}"] & \text{chlorin-e}
\end{tikzcd}$$

The metal complex of chlorin-e, copper chlorin-e, was made and rendered water soluble in the same way as the metal-free chlorin-e. The copper atom is bound in the center of the molecule as magnesium is found in chlorophyllin.

Pyrroporphyrin is a chlorophyll porphyrin obtainable by alkaline cleavage of pheophytin at higher temperatures. It is a mono-carboxylic acid retaining the original propionic acid group of the chlorophyll molecule; it has one carbon atom in the nucleus which is saturated with hydrogen and not substituted. The water soluble derivative, pyrroporphyrin sulfonic acid was prepared by sulfonation of the carbon atom with pyridinium-N-sulfonic acid according to the method of Treibs.[8]

Deuteroporphyrin is a blood porphyrin with 2 propionic acid groups and with 2 unsubstituted nucleus carbon atoms. It is obtained by reduction of hemin with resorcinol following Schumm's[9] method. In order to make the compound water soluble, the di-sulfonic acid derivative was prepared by sulfonation with pyridinium-N-sulfonic acid (Treibs). The 2 sulfonic acid groups are bound to the unsubstituted nucleus carbon atoms. This deuteroporphyrin di-sulfonic acid, having 4 acid groups of which 2 are the original propionic acid groups of the deuteroporphyrin and 2 are formed by sulfonation, is water soluble. The tetra sodium salt was prepared.

The copper complex of this compound has the copper atom bound in the same way as the metal is bound in copper chlorin-e.

These compounds are readily identified in solution by their characteristic absorption spectra.

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[8] Treibs, A., *Annalen d. Chemie,* 1933, **506**, 196.

[9] Schumm, O., *Z. f. Phys. Chemie,* 1928, **178**, 11.

Compound	Absorption spectrum Maxima $\mu\mu$	Sequence of Intensities
chlorophyllin-a tri-sodium salt	639, 595, 530	I, II, III
chlorin-e tri-sodium salt	653, 598, 543, 503	I, IV, II, III
chlorin-e, copper tri-sodium salt	629, 585, 503	I, III, II
pyrroporphyrin sulfonic acid sodium salt	631, 575, 554, 514	III, IV, II, I
deuteroporphyrin di-sulfonic acid tetra sodium salt	627, 613, 572, 549, 512	III, V, IV, II, I
deuteroporphyrin copper disulfonic acid tetra sodium salt	573, 537	I, II

For further information concerning these compounds, the reader is referred to the original works of Hans Fischer or to the comprehensive reviews by Steele[10] and by Fischer.[11]

Method. Twenty-five mg amounts of the various compounds were dissolved in 100 cc portions of 4% glycerine broth or Sauton's medium in 250 cc Erlenmeyer flasks and the solutions sterilized in the Arnold for 45 minutes on 2 successive days. The pyrroporphyrin sulfonic acid was practically insoluble in broth and Sauton's medium after sterilization. The final pH of the media was determined by glass electrode. All pH values were between 7.15 to 7.60. The flasks were inoculated with young actively growing pellicles of avian or human-37 tubercle bacilli and incubated at 37°C in the dark for varying periods (21-42 days). Each compound was tested in a total of 12 flasks on three different occasions.

Results. Examination of the data shows that the chlorin-e and chlorophyllin-a definitely retarded the growth of both the avian and human strains. The inocula on the media containing these 2 compounds showed very slight filmy growths while the control cultures and those containing copper chlorin-e, deuteroporphyrin, copper deuteroporphyrin, pyrroporphyrin showed luxuriant growths covering the whole surfaces of the flask and encroaching on the side walls. In order to determine whether greater concentrations of the various compounds would have any effect, inoculations were made into media of 100 cc volume containing 50 mg of compound. The results were the same as those with the smaller concentration (See Fig. 1).

To test the viability of the bacilli which had been incubated in the chlorin-e and chlorophyllin flasks, transplants were made into control media not containing chlorophyll. Normal luxuriant growth of the avian strain occurred within 9 days and the human-37 showed good growth in 16 days, indicating that the compounds were bacteriostatic but not bactericidal in the concentrations used. Both organisms on

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[10] Steele, C. C., *Chem. Rev.*, 1937, **20**, 1.

[11] Fischer, H., *Chem. Rev.*, 1937, **20**, 41.

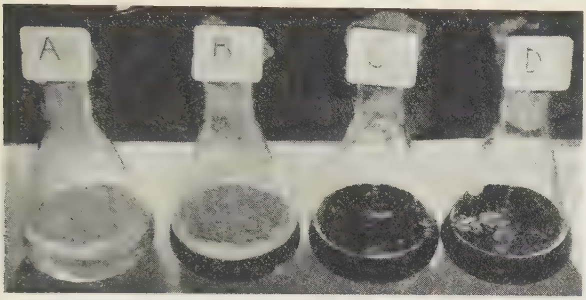

FIG 1.

Photograph shows amount of growth of *M. tuberculosis* strain H-37 on 4% glycerine broth containing:

A—No compound (control).
B—.025 % copper chlorin-e.
C—.025 % sodium chlorin-e.
D—.025 % sodium chlorophyllin-a.

all media retained their original morphological and acid-fast properties.

The pellicles assumed the color of the compounds on which they were grown (green in the case of chlorin-e and chlorophyllin and pink in the deuteroporphyrin). In order to determine in which way the pigment associates with the bacterial cell, the green pellicles were subjected to the extraction technics of Anderson[12] and of Heidelberger[13, 14] and collaborators. To ascertain whether the chlorophyll derivative was contained in the lipoid fraction of the bacillus, the green pellicles of H-37 grown on copper chlorin-e were, after washing with distilled water and drying, extracted with acetone, following Anderson's procedure, to remove the lipoids. Practically none of the color was removed with the lipoid extract, the organisms remaining green.

To facilitate the extraction of the proteins, the defatted organisms were ground in a ball mill until no bacilli remained intact, as determined by microscopic examination. The proteins were extracted by alkaline solutions, both N/10 and N/20 KOH removed the color completely. Addition of acetic acid to these alkaline extracts precipitates a green material which for purification may be redissolved in N/10 or N/20 KOH and reprecipitated by addition of acetic acid. The green compound gives a positive protein test with Millon's reagent. It has an absorption spectrum, the bands of which, compared to those of aqueous solution of copper-chlorin-e-sodium are shifted to the red end of the spectrum for approximately 10$\mu\mu$. This phenomenon of shifting of the absorption bands for 10 to 15$\mu\mu$ towards the longer wave lengths is a typical and well recognized property of chlorophyll protein complexes as has been shown by Smith, French and earlier investigators. As a result of the observations of these authors, the union of chlorophyll and protein is already well recognized as a naturally existing combination, chlorophyll of higher plants (Smith[15]), bacterio-chlorophyll of purple bacteria (French[16]).

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[12] Anderson, R. J., *Physiol. Rev.*, 1932, **12**, 172.

[13] Heidelberger, M., and Kendall, F. E., *J. Exp. Med.*, 1931, **54**, 513.

[14] Heidelberger, M., and Menzel, A. E. O., PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 631; *J. Biochem.*, 1934, **104**, 655.

Conclusions. Chlorin-e and chlorophyllin in concentrations of .025 and .050% in glycerine broth and Sauton's media inhibited the growth of H-37 and avian tubercle bacilli. Copper chlorin-e, deuteroporphyrin, copper deuteroporphyrin, and pyrroporphyrin sulfonic acid-sodium salt showed no growth retarding effects in concentrations of .025 and .050%. Evidence is presented to suggest that the H-37 tuberculo-protein of the living bacillus is capable of binding copper chlorin-e. The absorption spectrum of the copper chlorin-e-bacillary protein complex when compared with that of copper chlorin-e-sodium in aqueous solution lends support to the assumption that chemical union between protein and pigment exists.

10805

Mosquito Transmission Experiment with Poliomyelitis Virus.

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Although a few attempts have been made to transmit the virus of acute anterior poliomyelitis by mosquitoes, the field does not appear to have been entirely exhausted of possibilities and the following is submitted as a slight contribution to this line of investigation. Simmons, Kelser and Cornell[1] have summarized the earlier efforts and this summary will not be repeated. Theoretical arguments for or against the possibility will decide nothing, but repeated efforts may add weight to either side of the question.

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[15] Smith, E. L., *Science*, 1938, **88**, 170.

[16] French, C. S., *Science*, 1938, **88**, 2272; *J. Gen. Physiol.*, 1937, **20**, 711; *ibid.*, 1937, **21**, 71.

[1] Simmons, J. S., Kelser, R., and Cornell, V. H., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 496.

The following is a brief account of the essential points of the experiment:

A normal *Macacus rhesus* monkey was inoculated intracerebrally with 0.3 ml of the Aycock strain of poliomyelitis virus on February 23, 1939. Forty-eight hours later (Feb. 25) this animal was in turn exposed to cages of *Aedes egypti* and *Culex pipiens,* both lots having been laboratory reared. Fifteen of the *Aedes* fed; none of the *Culex*. On the sixth day after inoculation (March 1) the monkey had a temperature of 104°F; the next day 105°F, and paralysis appeared on the eighth day after inoculation (March 3). This animal progressively grew weaker, paralysis spread, it died and was autopsied on the ninth day after inoculation. Eight to nine *Aedes* fed on this animal on the first day of illness (March 1; 6th day after inoculation) and on that day one *Culex* also fed. On the second day of illness (March 2) 16 *Aedes* and 8 to 10 *Culex* fed. On the third day of illness 6 to 7 *Aedes* and 6 *Culex* fed. On the fourth day of illness (March 4; 9 days after inoculation) 17 to 19 *Aedes* and one *Culex* fed. All of these feedings were with the same lots of insects in the same cages and observations as to feeding were made immediately after exposure. In all, 46 to 47 *Aedes egypti* fed (15 prior to rise in temperature and 31 to 32 after rise in temperature); 16 to 18 *Culex pipiens* fed, all after rise in temperature of the monkey.

Beginning on March 9th, a normal *Macacus rhesus* monkey was exposed to the above mosquitoes, and again on March 13th, 17th, 22nd and 28th. These dates were, respectively 9, 13, 17, 22, and 28 days after the inoculated monkey first showed a rise in temperature. The number of insects feeding on these dates were: *Aedes,* 16 on the 9th, 21 on the 13th, 20 to 24 on the 22nd, and 32 to 34 on the 28th; *Culex*, 8 on the 9th, 5 to 6 on the 13th, 12 on the 17th, 6 on the 22nd, and 7 to 9 on the 28th. The totals of these latter feedings were *Aedes,* 89 to 93; *Culex,* 38 to 41. The minimum interval between initial feedings on the inoculated monkey and the normal monkey was 5 days; the maximum 12 days. During the time between the last feedings on the inoculated monkey and the last on the normal monkey there were 25 deaths of females in the *Culex* cage and a somewhat larger number in the *Aedes* cage. At the end of the experiment there remained alive 48 female *Culex* and 56 female *Aedes*.

These remaining female mosquitoes of both species were killed with chloroform, grouped together, ground in a small amount of Tyrode solution, the suspension passed through a "V" filter and three-fourths of a milliliter of the filtrate injected intracerebrally into a new normal monkey.

Both of the normal monkeys used for exposure to the possibly infected insects and inoculated with the filtrate were observed for 3 weeks and presented no symptoms of disease.

There is an obvious defect in the method used which was not realized until late in the experiment. Attention is called to it that it may be avoided in the future. This has to do with the uncertainty as regards the insects which fed upon the infected monkey and later upon the normal one. This criticism is more logical regarding the *Culex* mosquitoes used than in the case of the *Aedes* for in the former the discrepancy in the number feeding and the total present is greater. The reason for permitting this feature to enter the experiment was that previous attempts to induce the Culicides to feed in small groups, especially of females alone, had met with but scant success. These mosquitoes seem always to feed better in swarms, and especially in the presence of males. It would be advisable to so feed them upon the infected animal in the future, remove the females that have fed, then later create a new swarm with these and newly added males at the time of exposure to the normal animal.

Summary. 1. Of approximately 100 female *Aedes egypti,* 46 to 47 fed upon a *Macacus rhesus* monkey inoculated intracerebrally with the Aycock strain of poliomyelitis virus and later 89 to 93 of these females fed upon a normal monkey at dates ranging from 5 to 28 days after the feedings upon the inoculated animal following first symptoms. Similarly, of approximately 75 female *Culex pipiens,* at the same intervals, 16 to 18 fed upon the inoculated animal and 38 to 41 of the same original group fed upon the normal monkey. 2. At the end of the experiment the remaining female mosquitoes were chloroformed, ground, suspended in Tyrode solution, and the filtered solution inoculated intracerebrally into another normal monkey. 3. Neither of these normal animals, inoculated either by cerebral injection or exposure to supposedly infected mosquitoes, developed any symptoms of poliomyelitis in the 3 weeks period of observation.

10806 P

On the Administration of Heparin *Per os.*

ALBERT FISCHER AND TAGE ASTRUP.

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In view of the possibility of using heparin in the prevention of thrombosis any information concerning the physiology of that substance is of considerable importance. For the inhibition of the clotting of blood *in vivo* heparin is applicated by intravenous injection.[1] With reference to the chemical resistance of heparin and the reported excretion of it in the urine[2] it was thought possible to apply it orally.

Heparin with the activity k = 3,4 and prepared as described before[3] was dissolved in water (10 mg per cc). Mice were then given 0.5 cc from a syringe with a special glass tube inserted through the mouth in the stomach. Blood was obtained by cutting the tail. None of the mice in the experiments showed any delayed clotting of the blood, notwithstanding the great amounts of heparin given. There were, therefore, the following two possibilities: (1) Heparin is inactivated in the alimentary tract, or (2) it is not absorbed by the organism.

To prove which is the case, mice were given heparin in the same manner as before, and during the following 5 days the feces was collected at intervals. It was then extracted with 0.50 cc of water per 10 mg of feces, centrifuged and the supernatant liquid tested for inhibitory action in the usual manner. To 5 drops of chicken plasma one drop of the fluid was added, and the clotting time by the addition of a drop of chicken thrombokinase was observed in a water-bath at 39°.[4]

In Table I two of the 9 experiments are described. The chicken plasma clotted, with the addition of thrombokinase only, in 1½ min. The experiments show that the heparin passes the alimentary tract without deterioration. The feces of the control mice showed no delayed blood-clotting. Two of the heparin mice showed also no inhibitory action of the feces, but this might be due to vomiting of the heparin solution.

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[1] Murray, D. W. G., and Best, C. H., *J. Am. Med. Assn.*, 1938, **110**, 118.

[2] Wilander, O., *Skand. Arch. Physiol.*, 1939, **81**, Suppl., 48.

[3] Astrup, T., and Behrnts Jensen, H., *J. Biol. Chem.*, 1938, **124**, 309.

[4] Astrup, T., and Behrnts Jensen, H., *Skand. Arch. Physiol.*, 1938, **79**, 290.

TABLE I.

	Mouse A		Mouse B	
Day and hour	Feces, mg	Clotting-time min	Feces, mg	Clotting-time
1st 9^{00}	3	1½	45	1½
,, 15^{00}	47	>10	65	>10
2nd 9^{00}	13	>10	110	>10
,, 16^{00}	68	>10	84	1½
3rd 9^{00}	41	4	64	1⅓
5th 9^{00}	136	1½	132	1⅓

Heparin in mice is not absorbed from the alimentary tract by the animal organism, but is excreted undisturbed with the feces in the course of about 2 days.

10807

Coagulation Time of Blood in Normal and Adrenal-Demedullated Rats.

Dwight J. Ingle* and Warren C. Corwin.† (Introduced by F. D. W. Lukens.)

From the Wistar Institute of Anatomy and Biology, Philadelphia.

Vosburg and Richards,[1] von den Velden,[2] Cannon and Gray,[3] Cannon and Mendenhall,[4] and Mendenhall,[5] have presented evidence that adrenalinemia hastens the coagulation time of blood. Nice, Irwin and Kraft[6] observed that there was a decrease in the clotting time of blood following emotional excitation in normal rats and that this decrease was largely abolished by adrenalectomy. They concluded that this difference between normal and adrenalectomized rats was due to the loss of the adrenal medulla. In their experiment the samples of blood were obtained under ether anesthesia. Mendenhall[5] observed that coagulation processes were hastened by ether anesthesia.

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* Medical Research Fellow, The George S. Cox Medical Research Institute, University of Pennsylvania, Philadelphia.

† Instructor, Department of Pathology, Jefferson Medical College, Philadelphia.

[1] Vosburg, D. H., and Richards, A. N., *Am. J. Physiol.*, 1903, **9**, 35.

[2] Von den Velden, R., *Therap. Monatschr.*, 1911, **25**, 279.

[3] Cannon, W. B., and Gray, H., *Am. J. Physiol.*, 1914, **34**, 232.

[4] Cannon, W. B., and Mendenhall, W. L., *Am. J. Physiol.*, 1914, **34**, 243.

[5] Mendenhall, W. L., *Am. J. Physiol.*, 1915, **38**, 33.

[6] Nice, L. B., Irwin, O. C., and Kraft, R. M., *Am. J. Physiol.*, 1931, **96**, 305.

In this investigation we have compared normal and adrenal-demedullated rats in respect to changes in the clotting time of whole blood following ether anesthesia and following emotional excitation in the unanesthetized state.

Methods. Fifty albino male rats weighing 190-310 g were closely matched into pairs on a weight basis. One animal of each pair was subjected to bilateral enucleation of the adrenal glands by the method of Evans.[7] A minimum period of 60 days was allowed for the regeneration of the adrenal cortices of the operated rats.

The rat was emotionally excited by holding it on its back by means of rubber bands attached to each leg. The animal was then induced to struggle continuously by means of gentle probing with blunt instruments. In order to obtain a sample of blood the tail of the animal was placed against a cork board, a sharp razor blade was held against the tail and struck a light blow with a mallet. This served to excise the tip of the tail with a minimum crushing of the tissue. The tail usually bled freely and after discarding the first few drops a drop of as near a uniform size as possible was selected for observation. Coagulation times were determined by means of a Boggs' coagulometer. A minimum period of 2 weeks was allowed to elapse between experiments which involved the same animal. In each experiment a normal and a demedullated animal were run as pairs according to the original matching. The experimenter who made the observation on the coagulation time was not aware of the identity of the animal from which the sample of blood was taken.

In Experiment 1, 10 pairs of rats were subjected to emotional excitation for 10 minutes. Samples of blood were taken immediately before and immediately following the period of excitation. The average changes in the coagulation time of blood were small for each group and were statistically insignificant. In Experiment 2, 10 pairs of rats were subjected to emotional excitement for 20 minutes. The average coagulation time of the blood was decreased for each group but the changes were not statistically reliable. In Experiment 3, 10 pairs of rats were anesthetized with ether. Samples of blood were taken before the anesthetic was administered and 20 minutes after the beginning of anesthesia. A marked decrease in clotting time was found for both normal and adrenal-demedullated rats. The changes were statistically significant for each group. The group averages with the ratio between the difference and the standard deviation of the difference expressed as t. are summarized in Table I.

Adrenal-demedullated rats responded like normal rats in respect

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[7] Evans, Gerald, *Am. J. Physiol.*, 1936, **114**, 297.

TABLE I.
Coagulation Times of Normal and Adrenal-Demedullated Rats.
Each figure represents the average of 10 animals.

	Normal				Demedullated			
Experiment	Initial sec	Final sec	Diff. sec	t*	Initial sec	Final sec	Diff. sec	t*
10 min excitation	138.0 ±6	142.0 ±5	+ 4 ± 7.8	0.51	151.0 ±7.2	136.0 ±12.6	—15.0 ±14.4	1.04
20 min excitation	121.2 ±9	93.0 ±6	—28.2 ±11.4	2.5	108.0 ±3.6	91.2 ±8.08	—16.8 ± 8.8	1.9
20 min ether	136.2 ±.72	99.0 ±10.2	—37.2 ±12.3	3.02	138.0 ±6.0	76.2 ±11.4	—61.8 ±12.9	4.8

* A value for t of 3 or greater meets the usual requirements for statistical reliability.

to changes in the coagulability of blood following emotional excitation and following ether anesthesia. A considerable variability in normal values for coagulation time was noted. It is quite possible that the variability was due in part to experimental errors inherent in the technic used by us. This variability may have masked small differences in the response of our 2 groups of animals but we believe that marked differences in the coagulation time of blood cannot be produced in the rat by adrenal demedullation. This problem should be studied in larger animals, thus permitting the use of venous blood. Only then can the variations due to differences in thromboplastin content and rate of blood flow be eliminated. It would also be preferable to use one of the electrocoagulometers now available on the market.

Summary. Adrenal-demedullated rats responded like normal animals in respect to the coagulability of blood following emotional excitation and following ether anesthesia.‡

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‡ The authors wish to express their appreciation to Dr. E. J. Farris for the facilities provided them as guests investigators at the Wistar Institute of Anatomy and Biology.

10808

A New Method of Plethysmometry.

A. Kolin. (Introduced by H. Sobotka.)
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The method herein described is designed to eliminate some disadvantages of the standard plethysmograph, particularly the necessity of enclosing the object to be measured in a water-tight container through a tightly-fitting rubber cuff.* The new procedure makes it possible to determine volume changes in an open container. This is of particular interest in the clinical determination of blood flow through extremities where speed and ease with which the measurement can be performed are very desirable. The use of an open vessel offers an easy access to the extremity during the measurement and makes it possible to remove and reimmerse it within a short time, which is of advantage in experimental work.

The apparatus to be described can easily be assembled from standard parts available in any physiological laboratory. It requires a balance and a container large enough for the immersion of the object to be measured.

The principle upon which this method is based can be considered a supplement to the principle of Archimedes. According to Archimedes, an object immersed in a vessel of water loses weight equal to the weight of the volume of water which it displaces. Now it can be shown quite easily that the converse is also true namely, exactly the same amount of weight as lost by the immersed object is gained by the vessel in which it is immersed. The reason for this gain in weight is easily explainable as follows: In Fig. 1 we have a solid cylinder, C, immersed in a beaker of water, B, to the level, L. To simplify our considerations we may visualize in the liquid an imaginary rigid U-tube, as indicated in the dotted lines, having a cross section equal to that of the cylinder, C. It is evident that the liquid in our U-tube, up to the level L is in perfect balance. At level L the left arm of the tube is plugged by the lower surface of the cylinder C so that the column of water of the height h in the right arm of the U-tube presses upward on the lower face of the cylinder C with a force F_1, which is obviously equal to h times the cross section of the U-tube which product is in turn equal to the immersed volume of the cylinder between level L and the surface of the water, Ws. This force is represented by the vector F_1 and is identical with the buoyancy of Archimedes. Now, according to Newton's third law, there must be a second force F_2 equal to and opposing F_1 acting downward on the beaker of water so that it would apparently gain weight equal to that which the immersed cylinder loses. It is this force, F_2, which is utilized in the method presented here.†

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* For a discussion of the difficulties encountered with the standard plethysmograph, see Abramson, Zazeela, and Marrus, *Am. Heart J.*, 1939, **17**, 194.

Let us now imagine the beaker of water placed on one side of a balance and a human extremity partially immersed in it, but rigidly supported from the outside so as to avoid any contact with the moving parts of the balance, (as shown in Fig. 2). After establishing equilibrium we allow the part of the extremity immersed to increase in volume by an increment *n* cc, for instance by occlusion of venous return. This will disturb the equilibrium of the balance and we shall have to add *n* cc to a beaker of water on the opposite pan of the balance to restore equilibrium and bring the index pointer back to zero. This determination of the amount of water added to

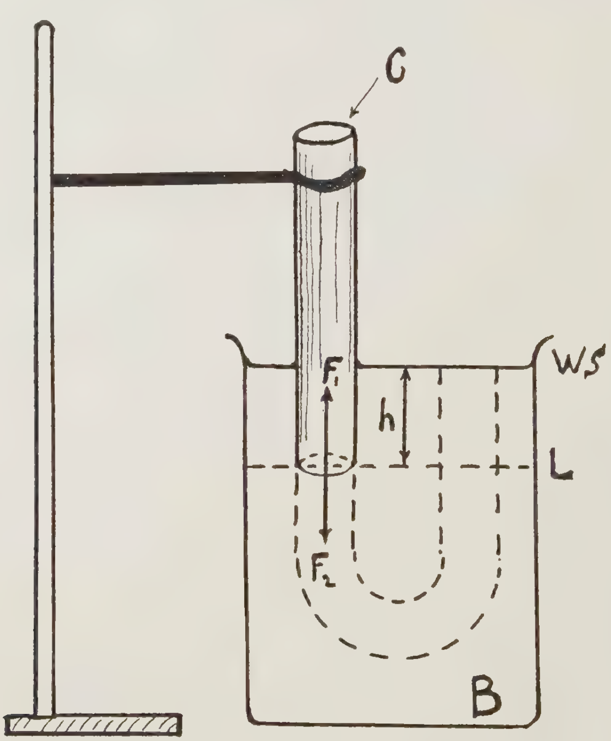

FIG. 1.
Discussed in text.

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† The above conclusions hold good for any arbitrarily shaped object immersed.

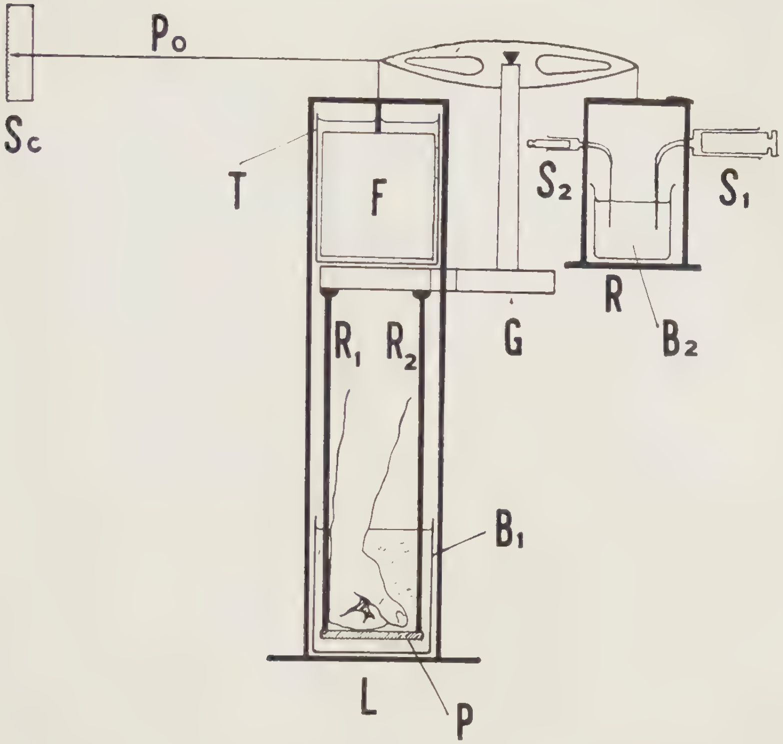

FIG. 2.
Scheme of the Plethysmometer.

Sc: Scale. Po: Pointer. S_1, S_2: Syringes. B_1, B_2: Beakers. F: Float. T: Buoyancy tank. R_1, R_2: Support rods. G: Base plate. L, R: Balance pans. P: Platform.

balance the scale constitutes the measure of the expansion of the extremity.

In the case of small volume changes it is also practical to read them off directly from the deflection of the pointer, after calibration by the addition of a known volume of water from the syringe S_2 (Fig. 2).

Figure 2 shows schematically the set-up used. The essential parts are the beaker of water, B_1, resting on the left balance pan, L, and the beaker of water, B_2, resting on the right balance pan, R. The arm is shown immersed in water in the beaker, B_1, being supported on a platform, P, which is rigidly attached to the ground plate, G, by means of stiff rods, R_1 and R_2. The arm is allowed to lean against R_1 and can, if desired, be rested against an additional support at the elbow for better fixation of position. An adequate weight is placed on the right pan, R, to maintain equilibrium of the balance. The finer adjustment of balance is accomplished by withdrawing or adding water by means of a large syringe, S_1. The small syringe, S_2, is calibrated in cc and is used to measure the amount of water required to restore equilibrium during the experiment, which amount measures directly the increase in volume of the arm when venous flow is obstructed. To increase the sensitivity we attached a long pointer, Po, the deflections of which were read on the vertical scale, Sc, or could also be recorded on a kymograph drum, if necessary.

For our purpose we needed a balance sensitive to about 0.05 g but strong enough to be loaded with 3.5 kg. By a simple means we made it possible to use a balance originally designed to carry a maximum weight of 1 kg. An air-filled float, F, of approximately the same volume as the beaker, B_1, was attached to the left side of the balance and immersed in a container of water, T, which was supported on the ground plate, G.‡ Its buoyancy just about compensated for the weight of the water in beaker, B_1. The addition of a small volume of water in beaker, B_2, was sufficient to balance the scale.

Even with an infinitely sensitive balance the sensitivity of our plethysmograph is limited by the following fact: If the immersed part of our object gains *n* cc in volume, the balance begins to descend on that side so that a part of the object will thus emerge from the water. Now, obviously, the motion of the balance in that direction could not continue beyond the point where *n* cc emerge from the water, since, in this new position of the balance, the same volume is submerged in water as before the expansion of the object; hence the balance ought to be again in equilibrium, if the restoring torque is negligible. Practically the latter is not the case and the deflection is somewhat smaller than the ideal maximum deflection. From this consideration it is clear that the amount of deflection per cc of volume change is inversely proportional to the cross section of the object at water level.

Due to the inertia of the system, our balance does not follow the rapid volume variations associated with the cardiac cycle, but is adequate for recording of variations which are slow as compared with the period of the balance. (4.3 sec in the example cited below.)

The effect of temperature variations on the density of water is negligible in the calibration.

To give an idea of the order of magnitude of the sensitivity of our set-up, we shall cite figures which were obtained in one typical arrangement. With a cylinder of 28 cm^2 cross section the deflection due to 1 cc volume change amounted to .9 mm. Since one cannot estimate the deflection on the scale closer than 0.1 mm, this constitutes the limit of resolution which corresponds to about 0.1 cc. The sensitivity could, of course, be easily increased by choosing a longer pointer or by reflecting a beam of light from a mirror mounted at the balance pivot.

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‡ Glycerol can be added to the water in T to obtain aperiodic damping of the balance.

We have made comparative measurements of blood flow through the hand using this method and the standard plethysmograph. The results were found to check well.

The author wishes to thank Dr. Zazeela and Mr. Marrus for their active coöperation in the physiological tests of the method.

10809

On the Toxicity of "Q" Substance from *Eberthella typhosa*.

E. W. DENNIS.

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Bruce White[1, 2] described an acid-alcohol-soluble "protein", extracted from *Eberthella typhosa* and other enteric bacilli, the presence of which was necessary for agglutination of specifically sensitized R bacilli. This substance, which he called "Q" substance, was antigenic but not species-specific. Anti-"Q" sera agglutinated p-forms of *Salmonella* bacilli; R-forms were agglutinated more slowly and to a lesser titer. S-forms contained "Q" but were inagglutinable by "Q" antisera. "Q" antisera gave heavy precipitates with solutions of "Q" substance. "Q" substance also gave a precipitate with normal serum but the specificity of the reaction with immune serum could be demonstrated by suitable dilution of the antigen. The "Q" substance could be differentiated from the P substances extracted by Furth and Landsteiner[3] by means of hot 75% alcohol. White reported "Q" substance as being non-toxic for rabbits, but the dosage was not stated.

E. typhosa, strain Ty 2, was grown on sodium-chloride infusion-agar, pH 7.4-7.6, for 24 hours at 37°C. The growth was washed from the agar with 3-5 cc of saline per Roux bottle. The bacilli were killed by the addition of one volume of pure acetone at 37°C for one hour. The killed flocculated bacilli were collected by centri-

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[1] White, P. Bruce, *J. Path. and Bact.*, 1932, **35**, 77.

[2] White, P. Bruce, *J. Path. and Bact.*, 1934, **39**, 529.

[3] Furth, J., and Landsteiner, K., *J. Exp. Med.*, 1928, **47**, 171.

fugation and extracted 3 times (18-24 hours) with 4 volumes of acetone at room temperature. The defatted bacilli were collected and dried *in vacuo* over calcium chloride; 28.5 g of bacilli (from 170 Roux bottles) were extracted for one hour at 45-46°C in 2 liters of .2N HCl alcohol (100 cc .2N HCl 1900 cc absolute alcohol). Extraction was carried out in a flask fitted with a reflux condenser. After extraction the bacteria were filtered off on a double layer of hard filter paper and washed with warm HCl alcohol. The washings were added to the extract, which was then chilled and made slightly alkaline (pH 7.8) with NaOH. An orange-colored precipitate appeared which was collected after ½ hour at 5°C. The precipitate was washed with cold absolute alcohol and with ether, and dried (weight, 1.462 g). Nine hundred and seven mg of crude "Q" was extracted with 40 cc of distilled water at pH 8, and centrifuged. The insoluble material was discarded. The clear, brown extract was chilled and .1N HCl carefully added to pH 5.5 when a heavy flocculent precipitate appeared. The precipitate was collected by centrifugation, dissolved in 10 cc dilute alkali and reprecipitated at pH 5.5. The recovery was complete. The final precipitate was washed in the neutral absolute alcohol and ether and dried. The final product weighed 147 mg. Qualitative tests on the crude "Q" substance showed the presence of a large amount of pentose, but the final product gave a negative Molisch test for carbohydrate. The Biuret test was strongly positive and Millon's test was positive after standing at room temperature. The substance was readily soluble in dilute acid, dilute alkali, and warm acid alcohol, but dissolved with difficulty in neutral water. This fraction, labeled Q_1W, showed on analysis: total N, 12.2%; total P, faint trace; reducing sugar after acid hydrolysis, faint trace; Sakaguchi's test for arginine grouping, positive.

Toxicity. "Q" substance dissolved in saline at pH 7.8-8 was injected intravenously into rabbits and intraäbdominally into mice. When injected into rabbits, 5 mg per kilo induced lethargy, rise in temperature, rapid loss in weight and diarrhea; 10 mg per kilo killed a 2 kg rabbit in 36 hours; 2 doses of 10 mg each, 24 hours apart, killed a 1.8 kg rabbit in 48 hours. Five milligrams of "Q" substance was lethal for 22 g mice. At necropsy the rabbits did not show any gross pathological changes which would account for death. The generalized hyperemia and hemorrhage which characterizes the action of the polysaccharide "endotoxin" was absent; microscopically the only findings were small foci of necrosis, without polynuclear infiltration, in the liver, in the Malpighian corpuscles in the spleen, in the lymph nodes and in the Peyer's patches.

Skin-toxicity. An attempt was made to produce the Shwartzman phenomenon,[4] using "Q" substance as a preparing agent. "Q" substance, in doses up to 1 mg, injected intracutaneously, produced within 18 hours, sharply circumscribed ulcers of the skin at the sites of injection (Fig. 1). There was little or no reddening prior to, and no hemorrhage subsequent to intravenous injection of 7.5 mg of endotoxin F/17A[5] which produced severe hemorrhagic necrosis at the site of injection of .001 mg of the same fraction. It was concluded that "Q" substance in saline solution is not capable of preparing the skin for the Shwartzman reaction.

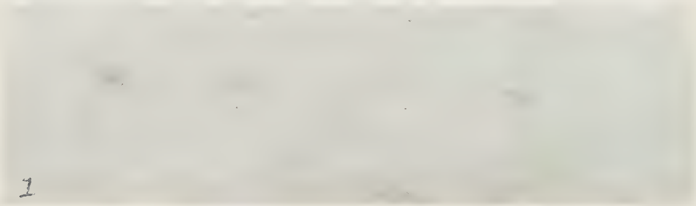

FIG. 1.
Skin-necrotizing action of typhoid "Q" substance in dose of 0.25, 0.1, 0.5, and 0.025 mg (read from left to right).

An experiment was then carried out to determine if the necrotizing action of "Q" substance could be neutralized by typhoid antiserum. The serum used was Felix's concentrated antityphoid immune-globulin solution, prepared at the Lister Institute. Aseptic precautions were taken throughout. The serum was diluted 1:25, 1:50; 1:100; 1:150; and 1:200. One cc of each dilution was added to each of a series of tubes containing 1 cc of 1:500 solution of "Q" substance, thoroughly mixed and the serum-antigen mixture incubated for 1 hour at 37°C. Precipitation, proportional to the concentration of serum, occurred in all tubes as well as in tubes containing undiluted horse- and rabbit-sera plus "Q" substance. The tube containing the highest concentration of immune globulin was centrifuged and the supernate and washed precipitate injected separately (Fig. 2a and e). The serum-"Q" substance mixtures were injected in .25 cc amounts into the left side of a rabbit. The right side of the rabbit was used for a parallel neutralization-test using Felix's serum against .01 mg doses of Shwartzman active polysaccharide F/20AE. Twenty-four hours after the endermal injections the sites of injection of the serum-"Q" mixtures were markedly inflamed but there were no ulcers. An eliciting dose of 7.5 mg of F/17A was injected intravenously.

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[4] Shwartzman, G., *J. Exp. Med.*, 1928, **48**, 247.

[5] Dennis, E. W., to be published.

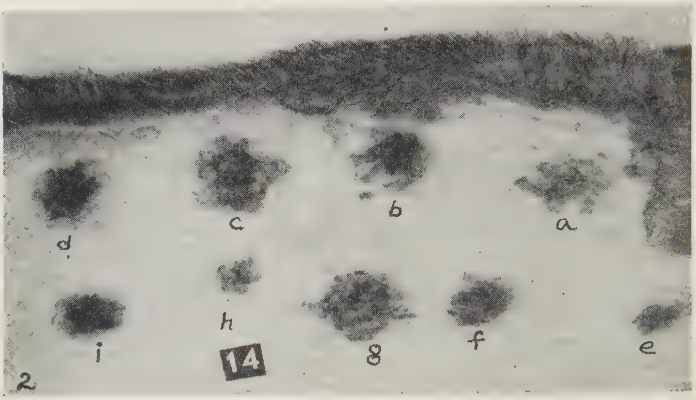

Fig. 2.

Shwartzman activity of serum-"Q" substance mixtures. Constant dose of 0.25 mg of "Q." Serum-dilutions: (a) 1:50; (b) 1:100; (c) 1:200; (d) 1:300; (f) 1:400; (e) Washed precipitate from (a); (g) Normal horse serum plus "Q"; (h) Normal rabbit serum plus "Q"; (i) Shwartzman active F/17A (.001 mg) plus normal rabbit serum, control.

The results are shown in Figs. 1 and 2. There was an intense Shwartzman reaction at all sites of injection, the intensity being proportional to the concentration of serum. There was no difference between the activity of normal horse serum-"Q" mixture and immune globulin-"Q" mixture. Removal of the precipitate reduced the intensity of the reaction (Fig. 2a); the washed precipitate was highly active (Fig. 2e).

Shwartzman[6] has reported the skin-preparatory activity of the specific serum-precipitates. However, the components and the serum-antigen mixtures were inactive; hence, the two phenomena are not analogous. Typhoid "Q" substance also precipitates dilute egg-albumen and normal human serum; skin-tests with the egg-albumen have been inconclusive but the precipitate with human serum is toxic. "Q" substance, in its property of precipitating complex proteins from solution, resembles the histones, but in its high solubility in alcohol it is different.

A specific "Q" antiserum, prepared by intravenous injection of 45 mg of "Q" substance into a rabbit over a period of 8 weeks, agglutinated both "H" and "O" suspensions of Ty 2 to a titer of 1:200 and gave a precipitate with 1:50,000 dilution of "Q" but did not prevent the necrotizing action of "Q"..

Conclusions. "Q" substance prepared from typhoid bacilli is lethal to rabbits and mice when given in adequate doses. We attribute its toxicity to its necrotizing action, which appears to be due to the action of "Q" in forming a toxic compound with serum-protein.

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[6] Shwartzman, G., Proc. Soc. Exp. Biol. and Med., 1931, **29**, 193.

10810

Isolation of Glutathione from Thymus Glands.*

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Glutathione, like the thymus extract, has been found to stimulate the growth and development of the offspring of treated rats.[1] Also its presence in the extract was indicated by glutathione determinations.[2] However, glutathione is believed to play but a subsidiary rôle in the activity of the thymus extract.[1] † The present communication deals with the presence of glutathione in the thymus gland by direct isolation.

Procedure. Two to four kg of calf thymus glands, obtained immediately after killing, were ground and deproteinized with 3 to 4 volumes of 5% CCl_3COOH. After filtration through infusorial earth under vacuum the filtrate was brought to about pH 6 with concentrated NaOH and refiltered. The mercury salt was precipitated with either mercuric acetate or mercuric sulfate.[3] It was separated, washed, decomposed with H_2S in the presence of N/10 H_2SO_4 and treated with CO_2 The cuprous salt was next precipitated according to Hopkins' method.[4] It was necessary to add the Cu_2O slowly in order to separate the grayish white precipitate of glutathione, which appeared first, from the red precipitate of purine-like material which appeared on the addition of excess Cu_2O. The glutathione was separated when no more grayish white precipitate was formed and before the red precipitate appeared. It was centrifuged, washed once with N/2 H_2SO_4 and then with water until the washings were sulfate-free. Several of these copper precipitates from different preparations were combined and reprecipitated with Cu_2O. The decomposed salt was evaporated in a vacuum desiccator over P_2O_5 to a heavy

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* This work was aided by grants from the Penrose Fund of the American Philosophical Society and the Rockefeller Foundation.

[1] Rowntree, L. G., Steinberg, A., Einhorn, N. H., and Schaffer, N. K., *Endocrinology,* 1938, **23**, 584.

[2] Schaffer, N. K., Ziegler, W. M., and Rowntree, L. G., *Endocrinology,* 1938, **23**, 593.

† An ether extract of the thymus extract has recently been found active.

[3] Kendall, E. C., McKenzie, B. F., and Mason, H. L., *J. Biol. Chem.,* 1929, **84**, 657.

[4] Hopkins, F. G., *J. Biol. Chem.,* 1929, **84**, 269.

syrup. It was placed in the refrigerator for several hours, stirred occasionally, and after it had crystallized was transferred to a porous plate for several more hours. The crystalline mass was ground in a mortar with 95% alcohol, filtered, washed with alcohol and dried *in vacuo*. This material gave a satisfactory analysis, but a low melting point (162°).

Further purification was obtained by precipitation of the silver salt[5] with silver lactate. Three more precipitations, copper, silver and copper, were made in the order given. This material was recrystallized by dissolving in water, filtering and placing in the refrigerator. After several hours when no crystals had formed, alcohol was added to the appearance of cloudiness. The next day the crystals were separated by filtration, washed with alcohol and dried. The melting point was 191.0° (corrected), that for glutathione[6] being 190°.

The material at the second copper precipitate stage gave the following analysis:

$C_{10}H_{17}N_3SO_6$	Calculated	N 13.68	S 10.44
	Found	13.26	10.72

Iodine Titration. 15.26 mg dissolved in 50 cc of N/12 HCl were titrated with N/100 iodine. Found 4.79 cc. For glutathione[7] 4.98 cc.

Specific rotation. Found $[\alpha]_{D}^{24.5} = -19.8°$ (2% solution in water)

For glutathione[6] $[\alpha]_{D}^{27} = -21.3°$

The material at the first copper precipitate stage in one preparation had a copper content of 16.6% (calculated 17.2%). It represented a yield of 0.28 g of glutathione per kg of thymus glands.

Summary. Glutathione has been shown to be present in the thymus gland by direct isolation.

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[5] Sullivan, B., and Howe, M., *J. Am. Chem. Soc.*, 1937, **59**, 2742.

[6] du Vigneaud, V., and Miller, G. L., *J. Biol. Chem.*, 1936, **116**, 469.

[7] Borger, G., Peters, T., and Kurz, M., *Z. physiol. chem.*, 1933, **217**, 255.

10811 P

Precipitation of Insulin with Rhodamine-B.

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A few years ago one of the authors in unpublished results showed that rhodamine-B would precipitate pepsin from solution. Since the well known proteolytic ferment precipitating agent, safranine, also precipitates insulin,[1] it was decided to determine if rhodamine-B would precipitate insulin and if so, whether the dye could be removed without inactivation of the insulin. Our experimental results show that insulin is readily precipitated by rhodamine-B at pH 7.2. The complex is soluble in distinctly acid solution, probably with decomposition into its components, for the dye can be practically completely removed by extraction with isoamyl alcohol, leaving the insulin behind in the aqueous layer.

To 1 cc of insulin, Lilly, (40 units per cc) 1 cc of a phosphate buffer solution of pH 7.2 was added, followed by 1 cc of a saturated solution of technical rhodamine-B which was either centrifuged or allowed to stand for several days to remove water insoluble impurities. Almost immediately after addition of the dye a flocculent precipitate separated out. After standing for approximately 10 minutes the precipitate was removed by centrifuging and washed with approximately 3 cc of the buffer solution. The washed precipitate was then dissolved in 1 cc of 0.1N HCl and diluted to 4 cc. Typical blood sugar changes following its subcutaneous injection into rabbits are shown in Table I. Although the number of animals used was not sufficient to give accurate data on the percentage recovery they do show, however, that it is reasonably satisfactory.

In other experiments, after the insulin-rhodamine-B complex was dissolved, the solution was extracted several times with isoamyl alcohol to remove the rhodamine-B. These extractions left a faint pink color in the aqueous layer which we were unable to remove by additional extractions. In order to remove the dissolved isoamyl alcohol the aqueous layer was extracted several times with anhydrous ether. The dissolved ether was removed by allowing the solution to stand at room temperature overnight. The volume was measured and the solution diluted so as to make it theoretically equivalent to 10 units of insulin per cubic centimeter. The blood sugar changes

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[1] Jacobs, H. R., and Ricketts, H. T., Proc. Soc. Exp. Biol. and Med., 1936, **35**, 473.

TABLE I.
Effect of the Various Preparations on Blood Glucose Concentration.

Theoretical insulin unitage per kg	Blood glucose per 100 cc, mg			Material used
	Before	After 2 hr	After 4 hr	
1.25	107	47	96	Rhodamine-insulin complex
1.25	121	78	113	,, ,, ,,
1.5	125	72	65	,, ,, ,,
1.5	120	75	81	,, ,, ,,
1.5	125	62	55	,, ,, ,,
2.0	111	48	86	,, ,, ,,
2.0	110	62	65	,, ,, ,,
2.0	109	50	61	,, ,, ,,
2.0	120	53	Convulsions	,, ,, ,,
3.0	108	49	Convulsions	Isoamyl alcohol extracted material
3.0	108	43	,,	,, ,, ,, ,,
3.0	111	60	75	,, ,, ,, ,,
3.0	111	60	83	,, ,, ,, ,,
3.0	115	47	Convulsions	,, ,, ,, ,,
1.5	112	56	75	Insulin, Lilly
2.0	120	64	68	,, ,,
2.0	124	43	Convulsions	,, ,,
2.0	118	84	,,	,, ,,

following its subcutaneous administration to rabbits are shown in the table. It will be seen to possess marked insulin activity.

While this work was in progress articles by Lasch and Schönbrunner[2, 3] came to our attention in which it was shown that rhodamine, as well as a few other substances, will protect insulin against digestion by trypsin. These authors have further shown that if insulin is mixed with dyes which will protect it against both tryptic and peptic digestion then its oral administration along with saponin leads to considerable insulin absorption. Experiments along these lines using the insulin rhodamine-B complex are contemplated for the near future.

We wish to thank the National Youth Administration for aid in this work.

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[2] Lasch, F., and Schönbrunner, E., *Arch. f. Exp. Path. u. Pharmakol.*, 1936, **182**, 452.

[3] Lasch, F., and Schönbrunner, E., *Klin. Wochenschr.*, 1938, **17**, 1177.

10812 P

Gynecomastia Associated with Cirrhosis of the Liver.*

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(Introduced by E. M. Hall.)

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The occurrence of gynecomastia associated with cirrhosis of the liver was first noted by Silvestrini,[1] and more fully described in 1923 and in 1926. Corda[2] added the observation that testicular atrophy accompanied the condition. More recent Italian writers refer to the phenomenon as the "Silvestrini-Corda syndrome."

Some 65 cases have been recorded in the foreign literature, but none has been found in English. Silvestrini believed the enlargement of the breast to be due to some substance or substances carried in the collateral circulation, while Corda considered it to be of endocrine origin.

We have observed 8 cases since 1935, 5 of whom came to autopsy. Three of the group had sex hormone studies. The essential anatomic findings were similar in all, *viz.,* atrophic cirrhosis, ascites, testicular atrophy and gynecomastia. All of our patients were chronic alcoholics and mammary enlargement occurred in each instance after the onset of ascites.

Studies of androgen and estrogen values in the urine were undertaken, 6-day specimens having been collected. The first specimens were allowed to stand at room temperature with toluene added as a preservative. Later (in C.F., M.C., G.A., and A.S.) the samples were kept on ice.

Estrogen was determined by a modification of the Allen-Doisy method with counter-current ether extraction after 2-hour hydrolysis of the sample acidified with 5% H_2SO_4. The smallest amount producing estrus in spayed rats of uniform weight was compared with the effect of crystalline estrone. Results of determinations were computed as gammas for 24-hour specimens.

Androgen extraction was done by a modification of Koch's method with 15-minute hydrolysis of the sample acidified with 5% H_2SO_4 and extraction with ether instead of benzene. Results of comb growth were compared with those obtained by the use of crystalline

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* Aided by a gift from Ethel Mossman Jacobs.

[1] Silvestrini, R., *Riforma Med.*, 1926, **42**, 701.

[2] Corda, L., *Minerva Med.*, 1925, **5**, 1067.

TABLE I.

		Estrogens (gamma)		Androgens (I.U.)	
	Age	Free	Total after hydrol.	Free	Total after hydrol.
			Normal Sex Hormone Values in Male Urine.*		
11-13, Dorfman[3]			1-5		1.1-15
20 adults, age 22-30 (our data)			10-12		20-82
			Sex Hormones in Gynecomastics.*		
J.M.	12		9		32
F.R.	52		60		36
C.F.	55	10.5	10.5	0	
			Controls.		
M.C.	26	4.5	12	20	32
G.A.	58	0	3	0	8
A.S.	48	0	1.2	0	8
M.	66		15	14	85

[3] Dorfman, R. I., Greulich, W. W., and Solomon, C. I., *Endo.*, 1937, **21**, 741.

* All data computed for 24-hour specimens.

androsterone and computed as international units for 24-hour specimens.

Later in the work, as noted in the tables, the free fraction was determined by the same procedure except that hydrolysis was omitted. The amount obtained by hydrolysis was called the total, which included the free and combined form.

Controls used were one individual with acute hepatic necrosis (M.C.), 2 with atrophic cirrhosis and ascites without gynecomastia (G.A. and A.S.), one with unilateral gynecomastia (M.), and a group of normal men, age 22 to 30 years.

The results are significant in 2 respects. First, 2 of the gynecomastics had increased estrogen (J.M. and F.R.), and second, the last (C.F.), and the individual with acute liver damage (M.C.) had 4.5 to 10.5 gamma estrogenic substance in the free form.

The only available data (Peterson[4]) indicate that negligible amounts of the free form, *i. e.*, without hydrolysis, are found in the urine of healthy men.

The rôle of the liver in inactivating estrogen is shown by Israel, *et al.*,[5] who found that estrone in the perfusion fluid in a heart-lung-liver preparation disappeared in 15 minutes. With the liver removed from the preparation all the estrone was recovered by bioassay. Golden and Sevringhaus[6] found that if the ovaries of rats were transplanted to the mesentery, estrus failed to occur, but if the same ovaries were retransplanted into the axilla then estrus occurred normally.

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[4] Peterson, D. H., Gallagher, T. F., and Koch, F., *J. Biol. Chem.*, 1937, **119**, 185.

[5] Israel, S. L., Meranze, D. R., and Johnston, C. G., *Am. J. Med. Sc.*, 1937, **194**, 835.

[6] Golden, J. B., and Sevringhaus, F. L., PROC. SOC. EXP. BIOL. AND MED., 1938, **39**, 361.

Summary and Conclusions. 1. We have studied 8 patients with hepatic cirrhosis in whom gynecomastia occurred late in the disease, confirming the findings of Silvestrini, Corda and others. 2. In all of these cases bilateral gynecomastia was preceded by the development of ascites. 3. The histological changes in these hyperplastic mammae are similar to the changes seen in experimental estrone stimulation (growth of ducts). 4. Sevringhaus, Israel, *et al.,* have shown experimentally that the liver normally plays a rôle in the inactivation or destruction of estrogen. Accordingly, it is reasonable to suspect that organic liver disease may result in abnormalities in estrogen metabolism. The increased urinary excretion of total estrogen in 2 cases and of free estrogen in 2 cases tends to justify this assumption. 5. An explanation of the absence of gynecomastia in acute hepatic necrosis and in the majority of cases of cirrhosis is not as yet apparent. The effect of testicular atrophy upon androgen excretion also is not clear.

10813

Changes in the Parenchymatous Organs Produced by Artificially Induced Fever.

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The purpose of this experimental study has not been to investigate the pathological changes which could be produced by lethal doses of artificially induced fever, as has been done previously by Hartman, Jacobsen and Hosoi, Baldwin and Nelson, and a few others,[1] but to make a quantitative comparison between the organ changes occurring in fatal and non-fatal doses of fever, and to investigate the fate of these changes after recovery of the animal. In our series of experiments male adult rabbits of a healthy standard breed were used. Nine animals died after a rapid high rise of temperature induced by the diatherm or radiotherm in a short period of 30 minutes (Group

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[1] Hartman, F. W., and Major, R. C., *Am. J. Clin. Path.*, 1935, **5**, 392; Jacobsen, v. G., and Hosoi, K., *Arch. Path.*, 1931, **11**, 744; Baldwin, W. M., and Nelson, W. C., Proc. Soc. Exp. Biol. and Med., 1928, **26**, 588.

TABLE I.

		A Lethal Temp. 30-60′	B 109° 8-9 hr	C 107° 40 hr	D 106.5° 3 hr 6 times	E Nembutal control	F Healthy control
Heart	Hemorrhages	+	+++	+	+	0	0
	Fatty degeneration	+++	++++	+++	++	+	0
	Necrosis	+	++	+	0	0	0
Lung	Hemorrhages	+++	++++	+++	+	+	0
	Atelectasis	0	+	+	0	0	0
	Pneumonia	0	+	0	0	0	0
Liver	Hemorrhages	0	+	+	0	+	0
	Loss of glycogen	++++	++++	+++	++	+	0
	Fatty degeneration	+	++++	++	0	0	0
	Focal necrosis	0	+	+	0	0	0
Spleen	Congestion	+	++	+++	++	+	0
	Hemorrhages	+	++	+	+	+	0
	Follicular necrosis	+	+++	++	0	0	0
Kidney	Hemorrhages	++	+	+	0	0	0
	Tubular degen.	++	++++	++	+	++	0
	Glycogen infiltra.	+	++	+	0	0	0
Adrenals	Hemorrhages	+	++	0	+	+	0
	Lipoid depletion	++	++++	++	+	0	0
	Cortical necrosis	0	++	+	0	0	0
Testis	Atrophy	+	++	++	+	+	+
	Necrosis	0	+++	++	0	0	0

A); 9 animals succumbed to a fever of 108° to 109° in a time interval from 8 to 9 hours (Group B); 5 animals were kept at a temperature of 107° until death occurred after an estimated time of about 40 hours (Group C); and 5 animals were exposed once a week 6 times and killed by air embolus one week after the last exposure. Twelve animals served as controls, 6 of which received similar doses of nembutal as used in our fever experiments in order to study the effect of this drug (Group E).

The principal pathological changes in the parenchymatous organs have been grouped in Table I. The frequency of their occurrence in each experimental group is indicated by a plus sign: 1+ indicates a frequency from 5 to 25%; 2+ a frequency up to 50%, 3+ to 75%, 4+ to 100%. Because of the relatively small number of animals used, this was thought to be a more truthful index than the quotation of actual percentage figures.

The most important changes in the heart were scattered interstitial hemorrhages of the myocardium and severe degenerative processes of the muscle fibers. The cells lost distinctness of cross striations and intercalated discs, and with the Sudan stain a fine emulsion of fat droplets could be noted in many of the muscle fibers. Small focal areas of necrosis with hyalinization and fragmentation of muscle fibers could be observed in the animals exposed to prolonged lethal doses of fever. The principal changes found in the lungs were acute venous hyperemia and intra-alveolar hemorrhage. The arterioles and small bronchi appeared constricted, the small veins dilated. Varying degrees of atelectasis, sometimes severe but not widespread or complete, were present in Group B. In this group a small percentage showed development of pneumonia. In the liver evidence of hemorrhage or congestion was only slight. The most significant change was the complete depletion of the liver cells of glycogen in the animals exposed to lethal doses of fever. The protoplasm of the liver cells in Group B showed the typical coarse granular appearance of parenchymatous degeneration with numerous small fat droplets present in the cytoplasm. Scattered areas of patchy focal necrosis could be found in the groups exposed to longer lasting lethal doses of temperature. They were usually located in the mid-zone of the liver lobules and consisted of acidophil hyalinized cellular debris invaded by leukocytes and macrophages. The most characteristic change in the spleen was the necrosis of lymphocytes in the Malpighian follicles and in the splenic pulp in the animals of Group B. Marked congestion was present in the spleen of all fever animals and in the nembutal controls. The hemosiderin pigment in the pulp was slightly increased in the chronic fever animals. In the kidney, congestion with hemorrhage was present in all fever animals except in the recovery group, while degenerative processes of tubular epithelium were most marked in Group B. These consisted of irregular enlargement of the tubular epithelium with obstruction of the tube lumen, an increased desquamation of epithelial cells, and the formation of cellular casts. An interesting finding was the appearance of glycogen in the tubular epithelium of the animals with depleted liver glycogen—a histological evidence of fever glycosuria. The adrenals showed a marked depletion of lipoid in fatal doses of fever and the presence of extensive cortical necrosis, which was most marked in the group exposed to 109° over 8 to 9 hours. Small hemorrhages were present in all fever animals and some of the nembutal controls. Necrosis of germinal epithelium with edema and hemorrhage in the interstitial tissues was found in the testicles of the animals exposed from 8 to 40 hours to high fever. The nuclei of the germinal epithelium showed changes varying from simple pyknosis to complete lysis. Giant cells with dense peripheral nuclei and a hyaline cytoplasm were frequently present. Acidophilic cell debris filled the lumina of the tubules. The changes seemed to affect the spermatids first, the spermatocytes second, and the spermatogonia last. Frequently an intact layer of spermatogonia could be found in tubules in which all other elements were destroyed. The interstitial cells showed only a slight swelling with some vacuolar degeneration. The thyroids of all fever animals showed moderate to severe depletion of colloid with corresponding hyperplasia of the epithelium lining the acini. Examination of the thymus, lymph glands, striated muscle, and omentum showed small petechial hemorrhages which were most frequently present in Group B. Examination of the intestinal tract, pancreas, bladder, ureters, and the larger vessels showed no significant change.

In summarizing, we can therefore state that the most frequent pathological changes observed in animals exposed to fever were vascular phenomena: congestion and hemorrhage. These, however, were present also in some of the animals which received nembutal alone and may have been at least partly caused by the drug itself, although their prominence in Group B undoubtedly points to fever as an important etiological factor. Degenerative changes of the cytoplasm and cellular necrosis were present especially in those animals which suffered a temperature of 109° over several hours and were less frequent or even absent in animals which died after a short-lasting exposure with rapid excessive rise in temperature. This observation would indicate that some time must elapse before the cells of the various parenchymatous organs show evidence of damage, and that fever over a brief period is apparently not very harmful to those cells although it produces death of the organism from some other cause. The difference in the cellular changes occurring in animals exposed to fatal and non-fatal doses of fever is very striking. No parenchymatous necrosis or replacement fibrosis was observed in animals who survived repeated short-lasting exposures to 106.5°. Since this is the condition we are meeting daily in the fever therapy of the human, we may emphasize the rather encouraging conclusions which can be drawn from these observations.

10814

Calcium Creosotate: V. Nature of Phenols Eliminated in Urine.*

EDWIN J. FELLOWS. (Introduced by Edward Larson.)

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Investigation[1-4] of calcium creosotate appeared desirable on account of its admission to U.S.P. XI without published reports on its clinical and experimental behavior. Volatile phenols could not be detected in sputum of hospitalized patients or in expired air of rabbits after oral administration of calcium creosotate.[2] It has been found, however, that calcium creosotate phenols are eliminated in rabbit urine[1] and also in human urine[2] after oral administration. Although calcium creosotate is advocated as a urinary antiseptic no clinical or experimental data have been found in the literature to indicate that its administration by mouth produces a bacteriostatic urine. It seemed, therefore, that the ability of *calcium creosotate* to produce a bacteriostatic urine, and the *form* in which it is eliminated should be investigated; especially since Knapp[5] reported that

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* I wish to express my thanks to Dr. A. E. Livingston for the helpful suggestions received during these experiments.

[1] Fellows, *J. Pharm and Exp. Therap.*, 1937, **60**, 183.

[2] Fellows, *Am. J. Med. Sci.*, 1939, **197**, 683.

[3] Fellows, *J. Pharm. and Exp. Therap.*, 1937, **60**, 178.

[4] Fellows, *J. Am. Pharm. A.*, 1939, **26**, 609.

[5] Knapp, *Schweiz. Wchschr.*, 1911, **49**, 229, 245, 257.

creosote was not eliminated as free phenolic material but largely in the form of glycuronates and ethereal sulfates. Statements have not been found in the literature concerning the *nature* of the phenolic material appearing in urine after creosote administration. It appeared desirable to determine if creosote was eliminated, as such, in urine, particularly in view of experiments[4] which have suggested the possibility that certain of its constituent phenols might be more readily destroyed in the body than others.

Experiments first were carried out in which large single doses of calcium creosotate were given to rabbits and urine specimens withdrawn at 1-hour intervals for 10 hours. On examination all of these specimens were found to be devoid of bacteriostatic activity. Normal human subjects were given single doses of calcium creosotate varying from 2 to 8 0.25 g tablets. Also a group of 8 normal individuals, after control urine specimens had been collected, received 2 0.25 g tablets every 2 hours during the waking period for 3 days. Urine specimens were collected every 2 hours throughout the day. The plates poured from inoculated urine specimens collected during the period of drug administration failed to show evidence of diminished growth when compared with those poured from inoculated specimens before the drug was given. The method of Davis and White[6] was used in testing urine specimens for bacteriostatic action. The test organisms used were *S. aureus* and *E. typhi*.

The following experiments were carried out to determine the amounts of free as compared with the amounts of conjugated phenols appearing in urine after administration of calcium creosotate. Twelve male rabbits were maintained in metabolism cages on a constant daily diet and 24-hour urine specimens collected. The 24-hour urine specimens were measured and filtered. Two cc portions of the filtrate were treated with 15 cc of diazotized paranitraniline reagent and allowed to stand several minutes. Two cc of 2% acacia solution were added, the mixture was made alkaline by the addition of 10 cc of 7% NaOH solution and the volume adjusted to 100 cc with distilled water. A known creosote solution was treated in the same manner and the urine specimens compared with it in a colorimeter. The results obtained during these experiments have been summarized in Fig. 1. Milligrams of phenols, in terms of creosote, found in the 24-hour urine specimens have been plotted against days of excretion. The first part of the figure (A to B) shows the values obtained before administration of calcium creosotate. At B the first dose of calcium creosotate was administered to 8 of the 12 rabbits in the series. The drug was given orally in a solution containing either 0.25 g or 0.5 g of phenols. On the first day all but one of the animals receiving the drug were given the 0.25 g dose, the other received 0.50 g. The next day 6 of the animals received the 0.25 g dose and 2 received 0.50 g. This procedure of decreasing the number of animals receiving the 0.25 g dose and increasing the number receiving the 0.5 g dose was continued until the eighth day of drug administration (C) when all animals except one were given the 0.5 g dose. It is to be noted that only a very slight increase in the free phenols excreted could be detected during this period (B to C). These determinations were all made on *non-hydrolyzed* urine specimens. After a 3-day control period (C to D) calcium creosotate was again administered to the same 8 animals in the same manner as before but the urine phenol determinations were made on *hydrolyzed* urine specimens (D to E). The marked increase in phenol values, obtained *after hydrolysis* as compared with the values obtained for the *non-hydrolyzed* specimens clearly shows that the portion of calcium creosotate which is excreted in rabbit urine is present almost entirely in conjugated form. Marenzi and Banfi[7] as well as Barrac[8] have reported that histidine and other imidazoles

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[6] Davis and White, *J. Urol.*, 1918, **2**, 107.

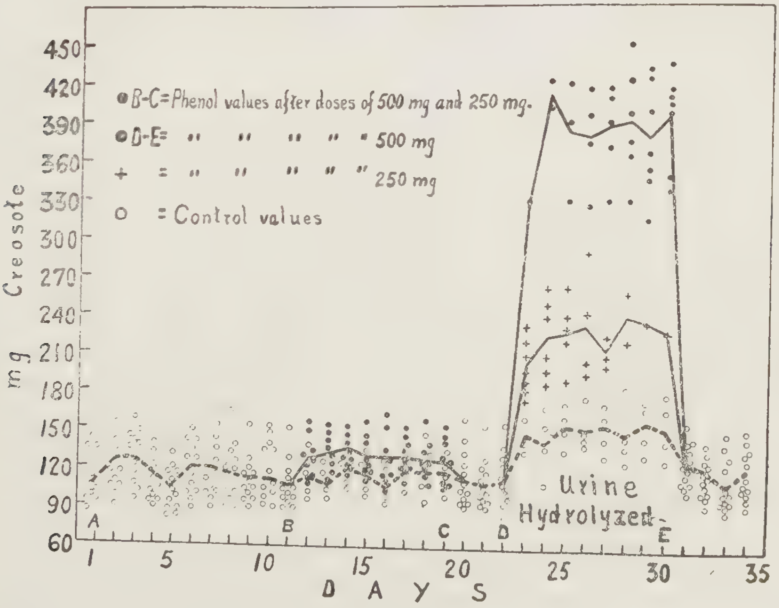

FIG. 1.

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[7] Marenzi and Banfi, *Rev. Soc. argentine biol.*, 1936, **2**, 62.

[8] Barrac, *J. pharm. Belg.*, 1936, **18**, 534.

react with the nitraniline reagent to give a color similar to that produced by phenols. For this reason direct reaction between urine and the diazo reagent is not entirely a measure of phenol content. In the present experiments it is to be emphasized that the animals were maintained on a constant weighed amount of food and therefore the amounts of non-phenolic constituents of the urine which react with the diazo reagent should be approximately the same during the time of drug administration as during the control period.

In previous experiments[4] evidence was obtained that a portion of the methoxy compounds of creosote were destroyed. It was suspected that perhaps the methoxy compounds might be more readily "oxidized" in the body than the other creosote phenols. If this happened the volatile phenols excreted in urine should exhibit analytical constants at variance with those for creosote. The following experiments, therefore, represent an attempt to determine if oral administration of creosote to rabbits would result in elimination of this mixture *per se* in urine.

Approximately 0.5 g of creosote (1.8% suspension in 0.5% acacia) was administered to each of 14 rabbits and 24-hour urine specimens collected. These specimens were pooled, acidified with HCl, heated to boiling and allowed to remain in a boiling water bath for 15 minutes. The urine was allowed to cool, saturated with sodium chloride, and then extracted with ethyl ether. This procedure was undertaken on each of 12 successive days. The combined ether extracts were allowed to stand over anhydrous sodium sulfate for several days, filtered and the ether removed by distillation. The residue of approximately 20 g was distilled in a 50 cc distilling flask and after traces of moisture were driven off it was found to distill between 192-215°C. Considerable solid residue remained in the distilling flask. This material was identified as benzoic acid. It probably arose from the hydrolysis of hippuric acid. The material distilling between 192-215°C was extracted with hot water and redistilled. As shown in Table I the boiling point of this material is somewhat lower than that of the creosote administered but previous experience[4] has indicated that creosote placed in aqueous solution, acidified and extracted with ether will boil in this range. It is to be noted that the specific gravity and methoxyl data correspond with those of the creosote administered. From these data it is apparent that after hydrolysis of the conjugates a portion of orally administered creosote can be recovered in rabbit urine. This indicates that the methoxy compounds are no more readily destroyed in rabbits than the other creosote phenols.

TABLE I.

Material	B.P. °C	Sp. gr., 25°C	MeO %
Creosote	200-215	1.085	12.5
Phenols extracted from rabbit urine after creosote administration	192-215	1.085	11.6

Summary. 1. Calcium creosotate administered either in single doses of 0.5 to 2.0 g or in amounts and at intervals approximating its therapeutic application (0.5 g every 2 hours) did not produce bacteriostatic urine in normal human subjects. 2. Evidence was obtained that practically all of orally administered calcium creosotate which is excreted in rabbit urine is present in conjugated form. 3. Data are presented to show that after hydrolysis of the conjugates a portion of orally administered creosote could be recovered in rabbit urine. This indicates that the methoxy compounds are not more readily destroyed in the body than the other phenols of the mixture.

10815

Some Effects of Testosterone on Sexual Differentiation of Female Albino Mice.

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Early investigators observed that the persistence of testicular transplants in oöphorectomized rodents produced hypertrophy of the clitoris. Since male sex hormone became available in crystalline form, it has been demonstrated that varying degrees of intersexuality may be induced experimentally in guinea pigs,[1, 2] mice,[3, 4, 5] rats,[6-10] and rabbits.[10] Although the previous findings of Raynaud indicated that a pronounced degree of masculinization of genetic

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[1] Dantchakoff, V., *Compt. Rend. Soc. de Biol.*, 1936, **123**, 823.

[2] *Ibid.*, 1937, **124**, 195.

[3] Lacassagne, A., and Raynaud, A., *Compt. Rend. Soc. de Biol.*, 1937, **125**, 351.

[4] Raynaud, A., *Compt. Rend. Soc. de Biol.*, 1937, **126**, 866.

[5] Raynaud, A., and Lacassagne, A., *Compt. Rend. Soc. de Biol.*, 1937, **126**, 868.

[6] Greene, R. R., and Ivy, A. C., *Science*, 1937, **86**, 200.

[7] Greene, R. R., Burrill, M. W., and Ivy, A. C., *Science*, 1938, **87**, 396.

[8] *Ibid.*, PROC. SOC. EXP. BIOL. AND MED., 1938, **38**, 4.

[9] *Ibid.*, 1938, **38**, 1.

[10] Hamilton, J. B., and Gardner, W. U., PROC. SOC. EXP. BIOL. AND MED., 1937, **37**, 570.

female mice resulted from both prenatal and postnatal administration of androgens, it was thought that more detailed and extensive studies on the mouse were justifiable.

The present work is based on dissections, wax reconstructions and histological studies of a total of 94 genetic female mice which displayed varying degrees of intersexuality. Forty-four adults received subcutaneous or intraperitoneal injections of testosterone propionate* between the time of copulation and parturition. Sixteen of these pregnancies were terminated by resorption or by the delivery of dead fetuses; 28 of the treated mothers produced a total of 74 viable offspring (Table I). Eighteen of the young (8 litters) which received prenatal treatment also received postnatal injections of 0.5 mg of testosterone at 48-hour intervals from birth until autopsy. Twenty offspring (7 litters) received only postnatal treatment, the testosterone being administered subcutaneously or massaged into the skin (Table I).

The prenatal administration of testosterone, if present in the organism at the period when both Müllerian and Wolffian systems are first differentiated, leads to the persistence of Wolffian duct derivatives and to the inhibition of the distal portion of the vagina. Five milligrams of testosterone administered to the mother on the fifth day of gestation did not influence the genital organs of the offspring (litter 13). A similar amount of testosterone injected on the seventeenth day of gestation (litter 3) did not maintain the Wolffian ducts in genetic female offspring nor prevent vaginal introitus, but did result in a mild modification of the clitoris. Animals receiving postnatal treatments (Table I) from birth until autopsy possess an enlarged clitoris dorsal to which is present a fold of skin surrounding the minute vaginal orifice; the genital tract is not otherwise appreciably modified. The most pronounced modifications are produced when the hormone is administered between the tenth and fourteenth days of pregnancy. There are, however, some variations among genetic females of the same litter even when large amounts of testosterone are administered at the critical period of pregnancy. In well affected offspring, subjected only to prenatal treatment, there is persistence of epididymides, ductuli efferentes extending from the rete portions of the ovaries to the epididymides, vasa deferentia, prostatic lobes, vesicular glands, Cowper's glands, and enlarged preputial glands. The clitori of such animals are hypertrophic, possess os priapi, cavernous bodies, and are perforated by penile urethrae. Except for their smaller size, the modified clitori of affected animals

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* We wish to acknowledge the generous supply of testosterone propionate (Perandren) provided by the Ciba Pharmaceutical Products, Inc.

TABLE I.
Summary of Animals Receiving Testosterone Treatment.

Litter No.	No. females in litter	Day of gestation	Amt of testosterone prenatal, mg	Postnatal treatment	Autopsy, days after birth
		(a) Prenatal Injection of Androgen.			
1	2	10	2	None	0*
2	1	10	5	”	0
3	3	17	5	”	0, 36, 143
4	4	10	5	”	1, 10, 30
5	3	12	2.5	”	0
6	4	10	1	”	0, 28
8	5	13	5	”	0, 10, 41
9	2	10	5	”	
		12	3	”	41
10	1	14	5	”	0
12	3	6	1		
		14	3	”	0, 20
13	3	5	5	”	0, 28
15	2	8	5		
		12	2.5	”	124
25	1	12	5	”	0
26	3	11	5	”	30
27	3	10	5	”	0, 60
28	2	12	5	”	0
29	3	12	10	”	10, 30
30	4	14	2	”	0
31	3	10	4	”	10, 15
32	4	10	5	”	0, 30
		(b) Prenatal and Postnatal Androgen.			
14	4	10	1	15 (0.5 mg)	30
17	3	10	2	9 ” ”	18
18	2	10	1	14 ” ”	28
19	1	5	5		
		8	2.5		
		10	2.5		
		13	2.5	40 ” ”	92
20	2	12	5	10 ” ”	21
21	3	9	5		
		13	2.5	10 ” ”	23, 112
22	2	7	5		
		10	2.5		
		12	5		
		15	2.5	40 ” ”	48
23	1	12	5		
		14	5	12 ” ”	30
		(c) Postnatal Androgen Only.			
11	1	None	None	22 ” ”	48
16	4	”	”	10 ” ”	21
33	5	”	”	30 ” ”	10, 20, 30
34	2	”	”	10 ” ”	10
35	3	”	”	10 ” ”	10, 30
36	3	”	”	Massaged	30
37	2	”	”	”	21

* “0” designates individuals autopsied within a few hours subsequent to birth.

are indistinguishable from the penes of normal littermate males. To date we have observed no indication of inhibition of the female tract other than the caudal vagina and the ovary. The ovaries of intersexes produced by prenatal androgen are abnormally small and are frequently anterior to the kidneys. We have observed no normal luteinization in the ovaries of our intersexed mice, although some were allowed to become mature (Table I, litter 15). Vesicular and non-vesicular follicles, in various stages of atresia, are frequent. In the 2 animals of litter No. 15, autopsied at 124 days of age, introitus did not occur, the uterine horns and proximal vagina were distended with fluid, epididymides persisted bilaterally, the vas deferens did not persist, and remnants of prostatic lobes were present. The ovaries of these 2 animals contained no corpora lutea but possessed a moderate number of vesicular follicles and many atretic ones. Cords of epithelioid cells penetrated the interfollicular areas. The uterine mucosa was metaplastic and consisted of a stratified squamous epithelium, the outer layers of which were highly keratinized. Many leucocytes had infiltrated into the lumina of the uterine horns.

Some of our animals received postnatal injections of androgen in addition to prenatal treatment (Table I). This stimulated the Wolffian duct derivatives and built them up to such degree that they equalled or surpassed the condition present in males of comparable ages. The 2 genetic females of litter No. 22 may be used as examples. Both individuals received prenatal testosterone on the seventh, tenth, twelfth and fifteenth days of pregnancy and 40 small doses (0.5 mg at each injection) of testosterone from birth until autopsy on the forty-eighth day postpartum. Both possessed atrophic ovaries consisting mostly of atretic follicles. The fallopian tubes were normal, ovarian bursae were absent, and there was no indication of pyometria in the uterine horns. The uterine horns fused to form a short duct which is apparently the cranial portion of the vagina. The vaginal canal opened into the prostatic urethra by means of a single orifice. The epididymides, ductuli efferentes, vasa deferentia, ejaculatory ducts, and vesicular glands persisted bilaterally. The prostatic lobes and Cowper's glands were distended by secretions. The penes were perforated for their entire extent by a penile urethra and they were histologically similar to the penes of males of similar ages.

Summary. Fifty-six genetic female mice have been examined subsequent to the prenatal administration of testosterone; 18 subsequent to both prenatal and postnatal treatment; and 20 subsequent to postnatal treatment alone. When testosterone is present in the pregnant mother, at a critical period, Wolffian duct derivatives persist and vaginal differentiation is inhibited. With sufficient testosterone treatment the clitoris is modified into a veritable penis and the utero-vaginal canal opens into the prostatic urethra.

10816

Quantitative Comparison of Responses of Isolated and of Intact Intestine to Seven Sympathomimetic Amines.

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Quantitative studies have been made concerning the isolated rabbit intestine inhibiting potency of the series of sympathomimetic amines which have been previously investigated with regard to inhibition of innervated and denervated intestinal fistulæ in unmedicated dogs.[1]

The motility of isolated segments of the duodenum and jejunum of rabbits was recorded by a modification of the technic used by Stewart.[2] The compounds studied were l-adrenalin (Parke, Davis & Co.), dl-arterenol (Winthrop Chemical Co.), cobefrin (Winthrop Chemical Co.), epinine (Burroughs-Wellcome & Co.), kephrine (Alba Pharmaceutical Co.), l-neosynephrin (Frederick Stearns & Co.), and dl-synephrin (Frederick Stearns & Co.). The effect on the isolated rabbit intestine of a certain dilution of adrenalin was determined, and a dilution of each of the other drugs was found that would duplicate the inhibitory effect of the test dilution of adrenalin. The test dilution of adrenalin was usually one part in 20 million or one part in 40 million. The compounds were tested in various orders and in some cases the entire series, except synephrin, was tested on the same intestinal segment.

Qualitatively, all of the compounds had effects on the isolated rabbit intestine similar to those resulting from adrenalin. There was inhibition of rhythmic contractions and a decrease in tonus. Hyperactivity was sometimes observed following the inhibition. The hyperactive recovery phase was most marked following neosynephrin, adrenalin and arterenol, and has been previously reported for the latter 2 compounds.[3]

The potency of the compounds as inhibitors of the isolated intestine relative to that of adrenalin is shown in Table I. The figures are given for the extremes obtained from comparisons on segments from nine rabbits, and each compound was assayed at least 8 times.

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[1] Youmans, W. B., Aumann, K. W., and Haney, H. F., *Am. J. Physiol.*, 1939, **126**, 237.

[2] Stewart, G. N., *J. Exp. Med.*, 1911, **14**, 377.

[3] Greer, C. M., Pinkston, J. O., Baxter, J. H., Jr., and Brannon, E. S., *J. Pharm. Exp. Therap.*, 1938, **62**, 189.

TABLE I.

Comparison of the relative potency of 7 amines as inhibitors of the isolated rabbit intestine with their potency as inhibitors of the dog intestine *in situ*. Figures indicate the increased concentration of the compound necessary to duplicate the inhibitory effect of a test concentration of adrenalin. The reciprocal of any figure in the table indicates the potency of the corresponding compound as compared to that of adrenalin.

	Formula	Isolated rabbit intestine	Intestinal fistulæ in unmedicated dogs
l-adrenalin	$(OH)_2C_6H_3 . CHOH . CH_2NHCH_3$	1	1
dl-arterenol	$(OH)_2C_6H_3 . CHOH . CH_2NH_2$	1½-2	1½-4
Cobefrin	$(OH)_2C_6H_3 . CHOH . CHNH_2 . CH_3$	3-5	2½-10
l-neosynephrin	$m\text{-}(OH)C_6H_4 . CHOH . CH_2NHCH_3$	4-10	25-100
Epinine	$(OH)_2C_6H_3 . CH_2 . CH_2NHCH_3$	10-20	10-25
Kephrine	$(OH)_2C_6H_3 . CO . CH_2NHCH_3$	20-50	25-100
dl-synephrin	$p\text{-}(OH)C_6H_4 . CHOH . CH_2NHCH_3$	500-1000	660-2500

The lower isolated rabbit intestine inhibiting potency of arterenol[3, 4] and neosynephrin[5] as compared with that of adrenalin has been previously reported.

Since the effect of a compound on the isolated intestine may be the reverse of that obtained in the denervated intestine *in situ* following an intravenous injection of the compound, some facts may be learned by utilization of both technics which may not be learned from one alone. Effects on the intestine *in situ* may be the result of reflexly liberated neurohormones and alterations in circulation produced by the injected compound. These factors are eliminated by the isolated intestine technic. However, the 7 compounds studied all had effects on the isolated rabbit intestine which were qualitatively similar to the effects on the innervated or denervated dog intestine *in situ*. With one exception there was correspondence both in the order of potency and the potency relative to that of adrenalin. Neosynephrin ranked higher as an inhibitor of the isolated rabbit intestine than as an inhibitor of the dog intestine *in situ*.

Conclusions. The similarity of the effects of 7 sympathomimetic amines on the isolated rabbit intestine and on the intact dog intestine indicates the physiological similarity of the smooth muscle used and demonstrates the peripheral and direct inhibitory action of these amines on the smooth muscle of the intestinal wall.

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[4] Barger, G., and Dale, H. H., *J. Physiol.*, 1910, **41**, 19.

[5] Boyd, E. M., *J. Pharm. Exp. Therap.*, 1937, **60**, 174.

10817

Cultivation of *Herpes febrilis* Virus on Agar Slant Tissue Cultures.

F. S. Cheever. (Introduced by H. Zinsser.)

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Since Löwenstein[1] reported the transmission of *Herpes febrilis* lesions from rabbit cornea to rabbit cornea in 1919 several methods of propagating the virus have been developed. Those most widely used today are the intracerebral inoculation of white mice (Andervont[2]) and inoculation of the chorio-allantoic membrane of a developing chick embryo (Saddington[3]). Tissue cultures methods as developed by Parker and Nye,[4] and Andrewes[5] while successful are more cumbersome than the two mentioned above. Recently Zinsser and his associates[6, 7] devised a solid medium consisting of Tyrode's solution, horse or beef serum and agar which used in conjunction with mouse or chick embryonic tissue gave large yields of different Rickettsiæ. It was suggested that this medium might be satisfactory for the cultivation of the herpes virus, particularly since FitzPatrick[8] and Kurotchkin[9] in this laboratory have reported success with the Eastern strain of equine encephalitis and with vaccinia respectively. This paper reports the successful propagation of the *Herpes febrilis* virus on this medium.

The virus used was one furnished through the kindness of Dr. F. P. Gay of New York City, neurotropic for both mice and rabbits, causing typical lesions on the chorio-allantoic membranes of embryo chicks, and propagated for some months in this laboratory by mouse brain passage. Infective material was secured by sacrificing a moribund mouse, removing the brain aseptically and grinding it in a mortar with 2 cc of broth and a small quantity of pyrex glass. The resulting suspension was centrifuged at 2500 rpm for 10 minutes and the clear supernatant fluid pipetted off. An intracerebral inoculation of 0.03 cc of this material invariably caused typical signs followed by death within 36-60 hours. Titrations carried out by making dilutions in broth averaged close to 10^{-3} as the final end-point. Finely minced chick embryo tissue was deposited upon agar slants prepared according to Zinsser's method. One 7-day chick embryo generally served to inoculate 4 tubes. Five drops of the virus suspension from a standardized Pasteur pipette (approximately 0.1 cc) were allowed to run over the tissue, and the tube incubated for 4 (rarely 3 or 5) days after being tightly stoppered. Transfers were effected by scraping off the tissue fragments of one tube and mixing them with a freshly minced chick embryo, which was subsequently inoculated upon 4 tubes—*i. e.,* each transfer represented at least a fourfold dilution of the original virus. To demonstrate the presence of virus the tissue fragments were ground in 2 cc of broth plus a small amount of pyrex glass, centrifuged, and the resulting supernatant fluid injected intracerebrally into mice in 0.03 cc amounts. In the course of several experiments during the past 2 months the presence of virulent virus has been demonstrated after 11 passages, covering over 6 weeks in the incubator. The virus originally titrated to 10^{-3}; after the eighth passage the end-point was $10^{-3.5}$, at which time the original virus would have been diluted over 10,000 times if no multiplication had taken place. The only difference noted between mouse brain passage and tissue culture passage was that in the latter case the incubation period in about one-third of the mice tended to be about 24 hours longer.

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[1] Löwenstein, A., *Münch. Med. Wochenschr.*, 1919, **66**, 769.

[2] Andervont, H. B., *J. Infect. Dis.*, 1929, **44**, 383.

[3] Saddington, R. S., Proc. Soc. Exp. Biol. and Med., 1932, **29**, 1012.

[4] Parker, F., Jr., and Nye, R. N., *Am. J. Path.*, 1925, **1**, 337.

[5] Andrewes, C. H., *J. Path. and Bact.*, 1930, **33**, 301.

[6] Zinsser, H., Wei, H., and FitzPatrick, F., Proc. Soc. Exp. Biol. and Med., 1938, **38**, 285.

[7] Zinsser, H., FitzPatrick, F., and Wei, H., *J. Exp. Med.*, 1939, **69**, 179.

[8] FitzPatrick, F., personal communication.

[9] Kurotchkin, T. J., Proc. Soc. Exp. Biol. and Med., 1939, **41**, 407.

As a comparative experiment tubes containing tissue killed by heating to 60°C for 30 minutes immediately prior to transfer on to the agar slants and parallel tubes containing live tissue were inoculated with equal amounts of the same virus suspension and incubated for 4 days before being injected into mice. Absence of tissue metabolism in the "heated tissue" culture tubes was evidenced by failure of the medium to assume an acid reaction, as always occurs in the presence of living tissue. Suspensions made from non-heated tissue fragments were invariably fatal for mice, those made from heated tissue fragments never caused demonstrable signs, the mice remaining healthy and not immune to subsequent intracerebral inoculations with a potent virus suspension. A series of "blind passages"—first generation on "heated tissue" tubes, second and subsequent ones on "non-heated tissue" tubes, have, after 3 generations, failed to show any evidence of the presence of the virus.

Summary. The virus of *Herpes febrilis* apparently persists and multiplies on agar slant tissue cultures prepared according to the method of Zinsser.

10818

Anticatalase Activity of Sulfanilamide and Related Compounds. IV. Peroxide Accumulation and Growth Inhibition in Pneumococcus.

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Sulfanilamide and structurally related compounds which have a free amino group in the position para to the sulfonamido group have been shown to have anticatalase activity.[1] The bacteriostatic activity of the compounds appears to depend upon this property.[2, 3, 4] An anticatalase, added to cultures of bacteria which form hydrogen peroxide as a metabolic product, interferes with its destruction, normally brought about by catalase. As a result, the concentration of peroxide around the organism may become sufficiently high to retard further growth. That sulfanilamide acts only indirectly as a growth-retarding agent by permitting the accumulation of hydrogen peroxide has been demonstrated for the Type I pneumococcus.[5] In the presence of sulfanilamide in concentrations of 8 to 10 mg % the amount of peroxide accumulated was such that its concentration per unit of growth was several times greater than in controls not containing sulfanilamide. Further evidence that hydrogen peroxide is the actual growth-checking agent was shown by the fact that sulfanilamide is unable to check growth of pneumococci in the absence of oxygen essential to the production of hydrogen peroxide.[6]

The development of a quantitative method[7] for determination of hydrogen peroxide in bacterial cultures has made it possible to show a quantitative relation between peroxide concentration and growth. Table I summarizes results of 4 typical experiments in which the peroxide concentration and amount of growth in broth cultures of the pneumococcus were determined at hourly intervals. The cultural conditions were the same as described before.[6] Growth was estimated turbidimetrically with $BaSO_4$ standards. The percentages

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[1] Main, E. R., Shinn, L. E., and Mellon, R. R., PROC. SOC. EXP. BIOL. AND MED., 1938, **39**, 272.

[2] Locke, A., Main, E. R., and Mellon, R. R., *Science*, 1938, **88**, 620.

[3] Locke, A., Main, E. R., and Mellon, R. R., *J. Immunol.*, 1939, **36**, 183.

[4] Mellon, R. R., *Modern Hospital*, 1938, **51**, 53.

[5] Shinn, L. E., Main, E. R., and Mellon, R. R., PROC. SOC. EXP. BIOL. AND MED., 1938, **39**, 591.

[6] Shinn, L. E., Main, E. R., and Mellon, R. R., PROC. SOC. EXP. BIOL. AND MED., 1939, **40**, 640.

[7] Main, E. R., and Shinn, L. E., *J. Biol. Chem.*, 1939, **128**, 417.

TABLE I.

Effect of Sulfanilamide on Growth and Hydrogen Peroxide Accumulation in Broth Cultures of Type I Pneumococcus.

Ten cc quantities of veal infusion broth containing 0.2% glucose were inoculated with 0.15 cc of 12-16-hour broth cultures. One cc samples were withdrawn at the intervals indicated, for estimations of growth and hydrogen peroxide content.

Exp. No.	5 hr			6 hr			7 hr			8 hr		
	G*	H_2O_2‖	A†	G	H_2O_2	A	G	H_2O_2	A	G	H_2O_2	A
a. C‡	18	10	0.6	26	15	0.6	70	20	0.3	70	21	0.3
S§	7	9	1.3	12	14	1.1	16	12	.8	21	23	1.1
b. C	15	11	.7	41	15	.4	80	21	.3	80	25	.3
S	7	<6	—	7	11	1.6	13	16	1.2	17	19	1.1
c. C	40	12	.3	70	18	.3	80	24	.3	80	26	.3
S	10	12	1.2	21	17	.8	21	22	1.0	21	34	1.6

*G = Growth. Growth was estimated by comparisons with $BaSO_4$ standards which had been calibrated against dilutions of a pneumococcus culture arbitrarily selected to represent 100% growth. The figures given are therefore growth units or percentages relative to the standard culture.

†A = Ratio, H_2O_2 concentration per unit of growth.

‡C = Control cultures containing no sulfanilamide.

§S = Cultures containing 10 mg% sulfanilamide added before inoculation as a 0.5% sterile solution in water.

‖H_2O_2 concentration in micrograms per cc.

reported in the table are in terms of a fully grown culture taken to represent 100% growth. Determinations of turbidities were made only after 5 hours' growth since the degree of turbidity developed before the fifth hour was too small to be accurately estimated.

For the estimation of hydrogen peroxide, 1 cc samples were withdrawn. To each was added 6 drops of a potato extract* and 2 drops of a 10% solution of *o*-tolidine in glacial acetic acid. The colors developed were compared with dilutions of a standard solution of malachite green oxalate and the peroxide concentrations were obtained by reference to a calibration curve made by plotting the dye concentrations against concentrations of peroxide which gave colors of equal intensity.

The amount of growth retardation is shown by a comparison of the growth, G, in the control cultures, C, with that in cultures, S, containing sulfanilamide. During the period of observation the growth was from 2 to 6 times greater in the controls than in the presence of sulfanilamide. Variations in the degree of stasis from experiment to experiment are to be expected, since the static action is readily affected by slight changes in the composition of the broth, temperature of incubation, pH, and size and age of the inoculum. The ratio,

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* Made by extracting 120 g potato with a solution composed of equal volumes of glycerol and a pH 7.0 phosphate buffer.

TABLE II.

Growth Inhibition and Hydrogen Peroxide Accumulation in Broth Cultures of Type I Pneumococcus Containing Sulfanilamide and Related Compounds.

Ten cc quantities of veal infusion broth containing 0.2% glucose were inoculated with 0.15 cc of 12-16-hour cultures. Samples withdrawn at intervals of 1 hr were tested for growth and hydrogen peroxide content.

Compound added	H_2O_2 concentration per unit of growth at			Relative therapeutic effectiveness*
	6 hr	7 hr	8 hr	
None	0.4	0.3	0.2	
Sulfanilamide	(1.8)	1.7	2.0	+
4, 4′-Diaminobenzenesulfonanilide†	(4.6)	1.5	2.0	+
2-(*p*-Aminobenzenesulfonamido)-pyridine‡	2.0	1.3	2.0	+
p-Acetylaminobenzenesulfonamide†	.6	.2	.2	0
None	0.6	0.3	0.3	
Sulfanilamide	1.1	.8	1.1	+
2-(*p*-Acetylaminobenzenesulfonamido)-6-aminopyridine§	1.5	1.5	1.6	+
4-Aminobenzenesulfonyl-4′-hydroxyanilide†	1.0	1.4	2.2	+
Sodium sulfanilyl sulfanilate‖	1.5	1.2	2.2	
N-(*p*-aminobenzenesulfonyl)-piperazine†	.6	.3	.3	toxic
N-(*p*-aminobenzenesulfonyl)-salicylamide†	2.2	2.8	2.7	+
None	0.5	0.3	0.3	
Sulfanilamide	1.2	.8	.7	+
p-Aminobenzamide	.5	.2	.3	0
p-Nitrobenzamide	1.8	1.4	.7	toxic
2-(*p*-Aminobenzenesulfonamido)-thiazole§	1.0	1.1	1.0	+
2-(*p*-Aminobenzenesulfonamido)-4-methylthiazole§	.8	1.1	1.1	+

*Therapeutic assays of these compounds will be reported in detail from this laboratory at a later date.

These compounds were furnished through the courtesy of Monsanto Chemical Co. (†); Merck and Co. (‡); Maltbie Chemical Co. (§), and Calco Chemical Co. (‖).

A, peroxide concentration per unit of growth, is in every instance higher in the presence of sulfanilamide than in its absence, and in the experiments cited was from 2 to 5 times greater.

The study of bacteriostatic action was next extended to include some structurally related compounds, both active and inactive. The results are summarized in Table II. For simplification, only the ratios, peroxide concentration per unit of growth, are given. Two types of control were used for each group of compounds, the one a culture containing sulfanilamide and the other a culture containing no added compound. Values in parenthesis are doubtful because of the slight degree of turbidity at the time of observation.

In the first group of compounds, 4,4′-diaminobenzenesulfonanilide and 2-(*p*-aminobenzenesulfonamido)-pyridine (sulfapyridine) showed ratios equivalent to those of sulfanilamide. *p*-Acetylaminobenzenesulfonamide, which is recognized as therapeutically inactive, caused no bacteriostasis and no increase in peroxide accumulation.

N-(*p*-aminobenzenesulfonyl)-piperazine, in the third group of compounds, showed no bacteriostatic activity and gave the same ratio as the control containing no added compound. The other compounds of this group may be considered to be about equivalent to sulfanilamide. While some of the ratios seem to be somewhat higher than those of sulfanilamide, the difference is probably of no significance because of the rather wide variations resulting from slight differences in experimental conditions.

The bacteriostatic activity of the thiazole compounds in the third group appeared to be about equivalent to that of sulfanilamide. *p*-Aminobenzamide, the carboxylic acid derivative corresponding to sulfanilamide showed no bacteriostatic activity. *p*-Nitrobenzamide showed marked activity.

Compounds which have been found to have therapeutic activity in pneumococcus or streptococcus infection in mice are indicated by +, in the last column of the table. Of the compounds tested so far in this laboratory, all of those which had bacteriostatic action, associated with increased accumulation of peroxide, were therapeutically active. Two compounds were too toxic in therapeutic dosage to be used in animal experiments. While it may be concluded that compounds with bacteriostatic activity have therapeutic effect, no prediction as to degree of efficacy in animal infection can be made, since many other factors must be considered in evaluating their usefulness as therapeutic agents.

The results demonstrate that there is increased accumulation of hydrogen peroxide in cultures containing compounds with anticatalase activity and that this accumulation of peroxide is associated with retardation of growth. Measurements of peroxide content is limited to that which has diffused into the medium from the immediate environment of the organism, where it may be present in concentrations many times greater than in the medium.

Summary. The effect of sulfanilamide, and other compounds capable of inhibiting catalase, upon growth and accumulation of hydrogen peroxide in broth cultures of the pneumococcus has been subjected to quantitative measurement. The concentration of peroxide per unit of growth was from 2 to 10 times greater in the presence of compounds which caused bacteriostasis than in control cultures.

10819 P

Effect of Cobalt on Erythropoiesis in Anemic Rabbits.

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During the past few years, in our experiments with different types of erythropoietic stimuli, the effect of cobalt on red cell formation appeared most impressive. Even such potent stimuli as excessive bleedings, or low oxygen tensions, do not evoke or maintain as great a reticulocyte response as does the injection of cobalt. It seemed advisable, therefore, to test the influence of cobalt on certain types of anemia.

In this report we shall describe the effects of cobalt on recovery from two types of artificially induced anemias in rabbits. One type was brought about by repeated bleedings and the other by injections of benzol. All animals were fed a normal diet consisting largely of Purina chow, lettuce and carrots throughout the entire experimental period.

In the first series of experiments 10 rabbits were bled approximately one-third of their calculated blood volumes on 3 occasions over a period of 2 weeks. This resulted in a fall of red cell count from 6.0-6.5 million per mm^3 to 2.5-3.1 million per mm^3. Corresponding to this drop in red cell count, the reticulocytes showed a sharp rise to 15-22%. On the third day following the reticulocyte peak, the animals were divided into 2 groups. Five animals were allowed to continue untreated, whereas the other 5 were injected daily with 50 mg $Co(NO_3)_2.6H_2O$. In the untreated group the reticulocytes disappeared from the circulating blood quite rapidly and reached normal values approximately 25 days later. The normal red cell count in these animals was attained in about 25-30 days. The anemic animals treated with cobalt, however, showed a marked reticulocyte response which was maintained at values of 8-10% or higher throughout the experimental period. The red cell counts in these cobalt-treated animals reached normal levels in about 15 days and continued to rise, reaching a value of 7.0-7.8 million per mm^3 10 days later.

In the second series, 12 rabbits were injected daily, for approximately 5 weeks, with gradually increasing amounts of benzol ranging from 0.5-1.0 cc. At the end of this period of treatment the red cell count had dropped from 6.0-6.6 to 3.5-4.5 million per mm^3. The reticulocyte percentage decreased to a significant extent, and the white cell counts were extremely low. From this time on, all the animals continued to receive daily injections of one cc benzol, but half of these, in addition, were injected daily with 50 mg $Co(NO_3)_2.6H_2O$. In the animals treated with benzol alone, the red cell count continued to drop, reaching a value of 2.5-3.8 million per mm^3 at the end of 50 days of treatment. Further injections of benzol into such animals generally resulted in death. In the animals receiving cobalt in addition to the benzol, the reticulocytes rose sharply from 0.5-1.2% to 10.4-13.8% within 2 weeks. This rise of reticulocytes was soon accompanied by an increase in the red cell count which attained almost normal levels at the end of 4 weeks.

In contrast to the severely hypoplastic condition of the benzol-treated animals' marrows, the marrows of cobalt-benzol injected rabbits showed marked hyperplasia, even greater than that observed in normal animals.

The marrows of normal rabbits treated with cobalt for 45 days show large numbers of megakaryocyte-like cells which appear to give rise to clusters of erythrogenic cells, in a manner comparable to that described by Jordan[1] in the yolk sac of the pig and in marrow of humans afflicted with typhoid. This would involve the formation of giant hemogenic cells or polykaryocytes from megakaryocytic elements by a direct division of the polymorphous nucleus. In the benzol-treated animals, where hemocytoblast development is inhibited, cobalt was found to markedly stimulate mitosis of the erythroblast and normoblast elements.

Summary. Cobalt injected into animals subjected to repeated bleedings or treated with benzol results in a rapid recovery from the anemia. Cobalt produces this effect by stimulating the formation of erythrogenic precursors in the bone marrow.

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[1] **Jordan, Harvey E., *Am. J. Anat.*, 1918, 24, 225.**

10820

Influence of Age of Animal and Nature of Injected Hormone Preparation on Antihormone Production.

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In the course of experiments dealing with the injection of various hormone substances, it became clear that the amount of antihormone produced was related to the age of the animal employed. In general, it appeared that adult rats, in response to injections of anterior pituitary-like gonadotropic hormones, produced greater quantities of antihormone substance and became refractory sooner than immature animals. This report is concerned with a description of results obtained recently in a detailed study of this phenomenon, along with some additional observations on the relative amounts of antihormone produced in response to 2 different anterior pituitary-like gonadotropic preparations.

Follutein,* a purified human pregnancy urine extract and Gonadin,* an untreated serum from pregnant mares, were the substances used in these experiments. Thirty-two immature Bartonella-free female rats (25-30 days of age) were divided into 4 groups. Daily subcutaneous injections of 5 R.U. Follutein were given to Group 1 for 35 days and to Group 2 for 95 days. Group 3 received daily injections of 5 R.U. Gonadin for 35 days and Group 4 for 95 days. Similarly, 28 Bartonella-free mature female rats (4-5 months of age) were separated into 4 groups and injected, as the immature animals, for 35 and 95 days with comparable amounts of the 2 hormone preparations. At the termination of the 35- and 95-day periods of treatment, the animals in each of the groups were subjected to light ether anesthesia, and the blood, drawn by cardiac puncture, was collected in tubes. The sera of the animals in each of the 8 groups were pooled and stored in a refrigerator during the course of the experiment.

These sera were tested for their antihormone content in the following way. Groups of immature female rats (25-28 days of age) were injected with 2.5 R.U. of either Follutein or Gonadin plus 0.2 cc of the appropriate serum daily for 5 days. The serum was

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* We are indebted to Dr. J. A. Morrell of Squibb and Sons for generous supplies of Follutein. We wish also to thank Mr. Donald Wonder of the Cutter Laboratories for supplying us with the Gonadin.

TABLE I.

Serum tested		No. of test animals	Mean ovarian wt ± S.E. (mg)	Range in ovarian wt mg
Follutein Groups				
A.	35 day injected, immature	15	30.8 ± 2.0	19-47
B.	95 ” ” ”	11	18.2 ± 1.0	13-25
C.	35 ” ” mature	12	14.9 ± 1.1	11-22
D.	95 ” ” ”	13	12.5 ± 1.1	7-18
E.	Controls	18	39.7 ± 1.5	27-50
Gonadin Groups				
F.	35 day injected, immature	13	31.3 ± 2.1	26-49
G.	95 ” ” ”	16	27.2 ± 1.6	18-35
H.	35 ” ” mature	12	25.5 ± 1.2	18-35
I.	95 ” ” ”	12	20.1 ± 2.1	12-32
J.	Controls	22	42.3 ± 2.7	26-69

S.E.—Standard error.

injected on one side of the body and the hormone on the other to prevent mixing. To serve as controls, one group of immature rats was injected with 2.5 R.U. Follutein and another group with 2.5 R.U. Gonadin daily for 5 days. On the 6th day, the animals were killed, the ovaries carefully dissected, freed of connective tissue, weighed immediately on a torsion balance and then fixed in Bouin's. The ovarian weights are given in Table I.

A number of conclusions are to be drawn from these data. (1) The serum of the injected adult rats possesses a greater inhibitory capacity than that of similarly treated rats which were immature when treatment was begun. This holds for both hormone preparations employed, the results being more pronounced with Follutein than with Gonadin. The serum of the young rats injected for 95 days shows greater protective power than that obtained from 35-day injected young animals, this result being more definite in the Follutein-treated animals than in those injected with Gonadin. The greater inhibitory effect of the serum from the 95-day injected young animals is probably due not only to the longer period of injection but also to a greater production of the antagonistic principle as the animal becomes older. (2) Although Follutein and Gonadin, in the dosage employed, produced approximately the same increase in ovarian weight in the various serum donor animals, the results indicate that more antihormone substance is formed for the pregnancy urine extract than for the pregnant mare serum preparation. As can be seen, this is true for both the immature and mature injected animals.

Histological examination of the ovaries from the animals employed to test the antihormone capacity of the anti-Follutein and anti-

Gonadin sera showed the same general picture after the 5 days of treatment. Both mature follicles and corpora lutea were seen.

It was now important to see whether the degree of antihormone activity of the serum could be related to any of its immunological properties such as its ability to form precipitins. Precipitin tests on the sera obtained from the 95-day injected animals were carried out as follows: 1-2, 1-5, and 1-10 dilutions† of the Gonadin preparation (1 cc contains 50 R.U.) and the original concentrated Follutein (1 cc contains 500 R.U.) were prepared, using 1% saline as the diluting agent; 0.1 cc of each of these dilutions was placed on a slide and alongside each, 0.05 cc of the different undiluted sera was added in such a way as to just allow the 2 drops to make contact. Macroscopic and microscopic examinations of the different preparations for precipitin formation were made after they had been allowed to stand for one hour. The relative amounts of precipitin formed were recorded and the results are shown in Table II.

Considering first the sera obtained from the Follutein-injected animals, it is seen that serum D which possessed greater antihormone capacity than serum B, showed a greater precipitin reaction. Similarly, for the Gonadin-injected animals, serum I which showed greater protective power than serum G, at the same time yielded more precipitins. Thus, it would appear that for either the Follutein or Gonadin-injected animals' sera, the greater the antihormone con-

TABLE II.

Dilution of Hormone Preparation.

Serum used	Full strength	1-2	1-5	1-10
1. Follutein				
B	++	++	+	+
B^1	0	0	0	0
D	+++	+++	++	+
D^1	0	0	0	0
Control*	0	0	0	0
2. Gonadin				
G	++++	++++	+++	++
G^1	++	+	+	0
I	+++++	+++++	++++	+++
I^1	++	++	+	0
Control*	+	0	0	0

Letters B, D, G and I refer to the untreated sera obtained from the experimental groups of animals mentioned in Table I. B^1, D^1, G^1 and I^1 refer to the same sera after precipitation with the non-specific protein in normal serum and urine.

0, +, ++, +++, ++++, +++++, indicate increasing degrees of precipitin formation.

*Control serum was obtained from untreated normal, mature female rats.

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† It was necessary to use these low dilutions of hormone because of the relatively low precipitin titers of the different sera.

tent, the greater the precipitin reaction. On the other hand, it is also to be noted that the sera obtained from the Gonadin-injected animals which possessed smaller antihormone content than those of the Follutein-injected rats, revealed significantly greater precipitin reactions.

It remained now to investigate to what extent non-specific factors participate in these precipitin reactions. Quantities of the sera obtained from the 95-day Gonadin-injected animals were precipitated with optimal proportions of normal horse serum, as determined by the method of Dean and Webb.[1] Similarly, portions of the sera from 95-day Follutein-injected rats were treated with optimal amounts of normal human urine extract made in the same way and in the same concentration as the pregnancy urine extract. After precipitation was complete, the mixtures were centrifuged, and precipitin reactions with the supernatant fluids again carried out as above. Account was taken of the extent to which the sera had been diluted in this process. The results, also indicated in Table II, show that the ability of the serum from the Follutein-treated animals to form precipitins is completely lost after absorption with normal human urine extract. Similarly, after precipitation with normal horse serum, the precipitin-forming power of the Gonadin-injected animals' sera is considerably reduced; they still, however, retain some capacity to form precipitins to Gonadin.

Despite a considerable amount of work on the subject, the question as to whether the protective substances which appear in the blood stream of animals treated with hormone preparations are immunological in nature or whether they are true antihormone substances in the sense used originally by Collip, has still not been definitely settled. In support of the former contention, Twombly[2] and Brandt and Goldhammer[3] have reported that pregnancy urine extracts, which had lost their gonadotropic activity through ageing or boiling, still were capable of inducing antihormone formation. Rowlands and Young[4] have shown that more antihormone is evoked in response to injections of a crude pituitary thyrotropic preparation than to a more potent purified thyrotropic extract prepared from the former. On the other hand, Sulman[5] has found that purified prolan, free from human urinary antigen, still induces antihormone but no antibody formation.

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[1] Dean, H. R., and Webb, R. A., *J. Path. and Bact.*, 1926, **29**, 473.

[2] Twombly, G. H., *Endoc.*, 1936, **20**, 311.

[3] Brandt, R., and Goldhammer, H., *Klin. Woch.*, 1938, **17**, 236.

[4] Rowlands, I. W., and Young, F. G., *J. Physiol.*, 1939, **95**, 410.

[5] Sulman, F., *J. Exp. Med.*, 1937, **65**, 1; *Arch. Int. Pharm. et Thér.*, 1939, **61**, 319.

In view of the fact that pregnant mare serum contains considerably more protein than the pregnancy urine extract employed, it would appear from our results that the amount of antihormone produced is not dependent solely on the total amount of antigenic material in the hormone preparation. This conclusion is to be reached from our finding that pregnant mare serum evokes the formation of less antihormone substance but more precipitins than a highly purified pregnancy urine extract. In fact, after absorption of the antiserum to Follutein with the non-specific antigen in normal human urine, its ability to form precipitins with the pregnancy urine extract is lost completely. In view of Sulman's experiments, this latter result indicates that the ability of pregnancy urine extract to induce precipitin formation is due to non-specific materials of the carrier substance. This non-specific factor must also operate in the case of the pregnant mare serum, although its capacity to form precipitins with the antiserum precipitated with normal horse serum is still slightly retained. In this connection, de Fremery and Scheygrond[6] have found that rabbits treated with serum of a non-pregnant mare failed to develop any inhibitory substance for the gonadotropic action of pregnant mare serum.

The reason why Follutein should evoke the production of more antihormone substance than Gonadin is not clear. It may mean that the gonadotropic complex in the former is a more powerful antihormone evocator than that present in the latter. The difference in antihormone production may also, in some way, be related to the relative proportions of gonadotropic factors (*e.g.,* FSH and LH) in the two preparations, since pregnant mare serum most likely contains more FSH than human pregnancy urine. It would be of interest to compare the amounts of antihormone principle formed in response to injections of relatively pure preparations of FSH and LH.

The results obtained in this study show that although true immunological reactions probably play a rôle in the formation of antihormone substances after injection of hormone preparations containing carrier antigenic material, other reactions not of the orthodox immunological type also may participate to some extent. Zondek and his coworkers[7] have also concluded that reactions not of the usual immune type operate in the production of antiprolan.

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[6] de Fremery, P., and Scheygrond, B., *Nature,* 1937, **139**, 1016.

[7] Zondek, B., Sulman, F., and Hochman, A., PROC. SOC. EXP. BIOL. AND MED., 1939, **40**, 96.

Summary. (1) Mature rats produce more antihormone principle in response to injections of anterior pituitary-like gonadotropic preparations than rats which are immature when treatment is begun. (2) Of 2 gonadotropic preparations tested, the one containing a large amount of protein evoked less antihormone production but more precipitins than the one containing less antigenic material.

10821 P

Pathology of B_6 Deficiency in the Rat and Response to Treatment with 2-Methyl-3-Hydroxy 4, 5-Dihydroxymethyl-Pyridine (Vitamin B_6).

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Vitamin B_6 deficiency in rats was first described by György.[1] From the macroscopic appearance of the skin lesions he coined the term "rat acrodynia". Many investigators have since confirmed György's description of the symptoms. This investigation was initiated in order to study the histopathology of this deficiency and the histogenesis of the changes effected by synthetic vitamin B_6.

Twenty-one-day-old rats were placed on the following diet:

	%
Cornstarch	68
Purified casein	18
Crisco	8
Salt mixture	4
Cod liver oil	2

supplemented with 40 micrograms each of thiamin and riboflavin and 0.5 mg of nicotinic acid. No attempt was made to supply factor II in this series of experiments. The characteristic dermatitis became evident after 6 to 9 weeks. The animals were maintained on the deficient diet for 72 to 86 days. By this time the symptoms were very severe: The paws were denuded, edematous and moist, the ears thickened and scaly, the snout swollen and some ulcers present under the tongue. Many of the rats showed roughness and redness of the surface of the lower abdomen.

Histological findings: At this stage the epithelial cells of the *ears* were somewhat larger than normal. The stratum granulosum was wider and instead of the usual 1 to 2 layers in thickness, extended over 4 to 5 layers. Hyperkeratosis was extreme. The stratum lucidum was also thicker and on its external side faded imperceptibly into the stratum corneum. Intercellular edema was striking and acanthosis was present. In places there was necrosis, especially at the tips of the pinna. Polymorphonuclear leucocytic infiltration appeared, particularly in the necrotic regions. Frequently serum or polymorphonuclear aggregates were present in the region of the stratum granulosum, giving the appearance of serous and purulent vesicles. Edema with polymorphonuclear leucocytes was also present in the subcutaneous tissues. The hair follicles and sebaceous glands were inconspicuous.

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[1] György, P., *Biochem. J.*, 1935, **29**, 741.

There was a considerable increase in fat and histocytes about the cartilage. This area was edematous and hypervascularized. The perchondrial connective tissue layers were not distinct and appeared as edematous loose fibrous tissue. In places the cartilage was in contact with the hypervascularized adipose tissue. There were areas at which there was distortion and apparent loss of continuity of the cartilage.

Other animals from the same series showing the same severity of symptoms were fed with a single dose of 100 micrograms of synthetic vitamin B_6. Twelve hours later the number of mitotic figures increased. In 24 to 48 hours round cells were present in the subcutaneous tissue and the edematous state was barely discernible. In 3 days to one week, all of the ulcerations except the extensive ones were healed and the hyperkeratotic tissue peeled off in the form of plaques, leaving a smooth somewhat pinkish epithelial covering. The perichondrial tissue also showed rapid organization with disappearance of edema and inflammatory reaction. The hair matrix showed activity with hair regeneration and the atrophic appearance of the sebaceous glands was lost.

The skin changes of the paws and snout were essentially the same as those described on the ears. On the abdomen and back the skin showed atrophic changes both of the epidermis as well as of the hair follicles. The intercellular edema was also pronounced. After the administration of the synthetic B_6 the reaction of the skin in these areas was similar to that found in the ears. The tongue ulcerations showed a tendency toward slow healing.

These results indicate the healing of the skin lesions due to B_6 deficiency, following the administration of synthetic vitamin B_6. The animals have not been on the diet sufficiently long to develop lesions due to factor II deficiency. Since our results make it possible to single out lesions due to B_6 deficiency, they are of aid in differentiating the remaining members of the B complex from B_6.

10822

Post-Parturitional Heat Responses of Newborn and Adult Guinea Pigs. Data on Parturition.*

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When newborn male and female guinea pigs are stroked on the posterior part of the back, they give a response which is similar to if not identical with that given by adult females at the time of heat.[1, 2] This behavior has been studied in order to ascertain the percent of newborn which give the response, the interval between birth and the time at which the response can be elicited, the length of time the response can be obtained, the effect of ovariectomy of the mother prior to parturition on the response and if the response is comparable with that displayed by the mature female.

The investigation required day and night observation of a large number of adult females at the time of parturition. Since little was known about the post-parturitional heat in the adult except that it is of frequent occurrence, data were collected bearing on the percent of animals which display post-parturitional heat, its length, the interval between the end of parturition and the beginning of heat, the ovarian condition in post-partum females which have not come into heat and the effect of ovariectomy prior to the end of pregnancy on the post-parturitional heat.

In addition the time required for parturition measured from the time of birth of the first young to that of the last, the interval between delivery of members of a litter and the time of day parturition occurred were recorded.†

The "heat response" of newborn animals. Animals in 84 litters composed of 93 females and 123 males were observed. Of these, 92 females and 121 males exhibited lordosis when fingered.

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* This investigation was supported by a grant from the Committee for Research in Problems of Sex, National Research Council.

[1] Young, W. C., Dempsey, E. W., and Myers, H. I., *J. Comp. Psychol.*, 1935, **19**, 313.

[2] Young, W. C., Dempsey, E. W., Hagquist, C. W., and Boling, J. L., *J. Lab. and Clin. Med.*, 1937, **23**, 300.

† Assistance in observing the animals at half-hourly to hourly intervals was given by Messrs. William R. Fish, Brewster Rundlett, and Arnold L. Soderwall. We are also indebted to Mr. William J. Spence, an animal dealer in Chatham, N. Y., for giving us access to his splendid breeding colony.

TABLE I.
Length of Interval Between Birth and Time the First "Heat Response" Was Obtained in Newborn Animals.

Time	92 females	121 males
Less than 15 min.	44	67
15 to 30 "	22	31
30 to 60 "	25	15
1 hr to 1.5 hr	1	7
2.5 "	0	1

The response was elicited almost immediately after birth (Table I), and, as in adult females, its intensity is greatest at the beginning of the period during which it can be obtained. This period averaged 2.4 hours in males, range 1.0 to 5.0 hours; and 2.8 hours in females, range 0.5 to 5.5 hours. Subsequently, it frequently can be obtained for several hours, but only if the animals are covered by the mother.

In order to determine if ovariectomy of the mother affects the behavior, 15 pregnant females were ovariectomized between the 50th and 55th day of gestation. Eight gave birth to normal litters between the 67th and 69th days of gestation. The 14 young of both sexes responded normally. Furthermore, normal responses were elicited from 13 young born to 5 females which did not come into heat or ovulate at the time of parturition.

Whether the response in young animals is true heat behavior stimulated by a hormonal action to which newborn are subjected *in utero* is not known. Quantities of injected estradiol benzoate (Progynon-B)‡ sufficient to cause a growth and differentiation of the vaginal epithelium similar to that described by Courrier,[3, 4] did not always induce the heat response. Of 22 spayed and normal animals injected with from 125 to 264 R.U. on the second or third day, only 12 gave the heat response when fingered. Furthermore, the 10 which did not display heat behavior following estradiol benzoate injections were subsequently injected with 0.4 I.U. progesterone (Proluton)‡ and did not respond to this hormone, although 20 R.U. estradiol benzoate followed 48 hours later by 0.1 I.U. progesterone is effective in 20-day animals. Obviously, procedures which induce heat on the twentieth day are ineffective in very young animals and until the "heat response" can be stimulated in newborn young with the regularity which is shown normally, we shall not know how to regard this post-partum behavior.

Post-parturitional heat in adult females. Fifty of 78 animals which were observed came into heat. This occurrence of heat post-partum in about two-thirds of the cases is less frequent than in non-pregnant animals. Thirty-one of the experimental animals had been observed during 124 reproductive cycles prior to pregnancy and heat occurred during 107 or 86.3%.

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‡ Supplied by Dr. Erwin Schwenk of the Schering Corporation.

[3] Courrier, R., *Compt. Rend. Soc. Biol.*, 1929, **99**, 24.

[4] Courrier, R., *Proc. Second International Congress for Sex Research*, 1930, 352.

Post-parturitional heat was shorter than normal heat. For 50 animals the length averaged 3.5 hours, range 0.5 to 10 hours. This compares with an average of 8.6 hours, range 4 to 15 hours, for 76 heat periods displayed prior to pregnancy.

The interval between the birth of the last young and the beginning of the post-parturitional heat is shown in Table II. In general, it was so short that a close correspondence between the beginning of parturition and the beginning of heat is suggested.

TABLE II.
Length of Interval Between the End of Parturition and the Beginning of Post-parturitional Heat in Adult Females.

Interval between end of parturition and heat in hours	0-1	1-2	2-3	3-4	4-5	5-6	6-7	7-8	8-9	9-10
No. of animals	13	17	6	4	3	2	1	3	0	1

The ovarian condition in animals which came into heat post-partum was determined by ovariectomy 20 to 45 hours later, and in 12 animals which did not come into heat by examination 24 to 44 hours post-partum. In the former ovulation had occurred. In 7 of the latter ovulation also had occurred, but in the remaining 5 it had not. The volume of the largest follicles in these ovaries was similar to that of follicles in non-pregnant animals immediately prior to ovulation,[5] but it is doubted that they would have ruptured because all showed signs of atresia and no evidence of luteinization was seen. These animals probably would not have come into heat until a new set of large follicles had developed.

The data suggest that post-parturitional heat in the adult is dependent on ovulation and is similar in this respect to heat in non-pregnant animals. An attempt was made to obtain critical evidence by observing the 8 females spayed between the 50th and 55th day of gestation which subsequently delivered normal litters. Of these, 4 were observed for post-parturitional heat without further treatment. Three did not come into heat. The fourth, however, displayed a normal heat of 4.5 hours' duration which began 4.5 hours after parturition. After heat the animal was killed, but no ovarian tissue was found. Heat in this case cannot be accounted for unless estrogens circulating in the blood were responsible or unless ovarian tissue was overlooked when the animal was killed.

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[5] Myers, H. I., Young, W. C., and Dempsey, E. W., *Anat. Rec.*, 1936, **65**, 381.

The former possibility is questioned because of results obtained from a test applied to the remaining 4 spayed animals. Within 45 minutes after parturition they were injected with 0.2 I.U. progesterone. None came into heat during the 11 to 25 hours they were observed. Had they been under the influence of estrogen, the progesterone should have induced heat.[6]

The possibility that ovarian tissue was overlooked is considered more likely, because of the failure of the above test and other observations. If no ovarian tissue was left behind in this animal, it was only one of 13 post-partum individuals in which heat occurred without ovulation. Furthermore, in none of 101 non-pregnant guinea pigs was heat without ovulation encountered.[7] Nevertheless, the doubtful case cannot be explained and until additional experimental animals can be established, the conclusion that the post-parturitional heat of adults depends on the presence of the maternal

TABLE III.
Length of Interval Within Which Parturition Occurred.

Length of interval during which birth occurred	No. of litters	Size of litters
45 min*	1	1 of 4 young
Less than 30 min	14	1 " 5 " 7 " 4 " 4 " 3 " 2 " 2 "
" " 20 "	10	2 " 4 " 5 " 3 " 3 " 2 "
" " 15 "	30	5 " 4 " 13 " 3 " 11 " 2 " 1 " 1 "
" " 10 "	16	1 " 4 " 6 " 3 " 6 " 2 " 3 " 1 "
" " 5 "	9	1 " 3 " 3 " 2 " 5 " 1 "

*30 minutes elapsed between the birth of the 3rd and 4th young. This was 14 minutes longer than the next longest of 44 recorded intervals and is not included in the averages shown in Table IV.

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[6] Dempsey, E. W., Hertz, R., and Young, W. C., *Am. J. Physiol.*, 1936, **116**, 201.

[7] Young, W. C., Dempsey, E. W., Myers, H. I., and Hagquist, C. W., *Am. J. Anat.*, 1938, **63**, 457.

TABLE IV.
Length of Interval Between Birth of Members of a Litter.

Order of young	No. of cases	Average interval and range in min
1st and 2nd	18	8.8, range 2 to 16
2nd '' 3rd	16	6.8, '' 2 '' 13
3rd '' 4th	9	5.5, '' 1 '' 12
4th '' 5th	1	14 min
Summary	44	7.4, range 1 to 16

TABLE V.
The Time of Day Parturition Occurred.

Time of day	12 mid.-1 a.m.	1 a.m.-2	2 a.m.-3	3 a.m.-4	4 a.m.-5	5 a.m.-6	6 a.m.-7	7 a.m.-8	8 a.m.-9	9 a.m.-10	10 a.m.-11	11 a.m.-12 noon	12 noon-1 p.m.	1 p.m.-2	2 p.m.-3	3 p.m.-4	4 p.m.-5	5 p.m.-6	6 p.m.-7	7 p.m.-8	8 p.m.-9	9 p.m.-10	10 p.m.-11	11 p.m.-12 mid.
Number of births	4	2	4	4	6	2	3	5	3	7	4	7	4	3	4	7	3	4	2	6	5	5	3	2

ovaries and ovulation must be advanced with reservations.

Data on parturition. The interval within which delivery of the young occurred was ascertained from observations of 80 litters (Table III). In Table IV the length of 44 intervals between the births of individuals are tabulated. The data are not sufficient to give significance to the progressive decrease in the average length of the interval between the birth of individuals, but the possibility of such a decrease is suggested.

The time of day parturition occurred was obtained for 99 animals and is summarized in Table V. The approximately even distribution throughout the 24 hours is surprising in view of the predominant nocturnal occurrence of heat.

Summary. Ninety-two of 93 newborn females and 121 of 123 newborn males displayed a response when fingered which is similar to the heat response of adult females. The behavior is independent of the post-parturitional heat in the adult and does not depend on the presence of the maternal ovaries. In the adult post-parturitional heat was displayed by a smaller percent of animals and was shorter than normal heat. The preponderance of evidence suggests that post-parturitional heat is induced by factors associated with ovulation in the maternal ovaries. The interval between delivery of the first and last members of a litter was less than 30 minutes in 79 of 80 cases. The time of parturition in 99 animals was distributed about evenly throughout the 24 hours of the day.

10823

Effect of Age upon the Cellular Phases of Skeletal Muscle.

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Preliminary to a quantitative study of the functional capacity of muscle at various ages, it was desirable to know the relative amounts of muscle cell and non-muscle cell tissue present at any given age. A fairly accurate quantitative approach to this problem can be had from calculations based upon the chloride and water concentration in muscle and blood serum. These calculations are based on the premise that all the tissue chloride is present in the non-muscle cell phases in concentrations approximating that of an ultra filtrate of blood plasma. The distribution of water between the muscle cell phase and non-muscle cell phase has been estimated from the data on rat's gastrocnemius muscle and blood serum using the equations as described by Hastings and Eichelberger.[1] In our calculations of the amounts of muscle cell and non-muscle phases and the concentration of water in the former, it is assumed that the tissue chlorides are associated with connective tissue having a water concentration of 80%. In these calculations the total neutral fat is assumed to be contained within the connective cells. Thus the total solids contained in 100 g of non-muscle cell phase is considered to be 20 g plus the amount of neutral fat found in 100 g of muscle.

The animals were killed by hemorrhage and the removal of blood from the tissues was aided by limb vein section and massage. The muscles were weighed after being dissected as free as possible from tendon and superficial fascia. The water content of tissues was determined by drying to a constant weight in an electric oven at 106°C. The neutral fat was determined according to the method described by Hastings and Eichelberger.[1] The blood samples were centrifuged under oil and the serum removed for analysis. The determination of the chlorides in muscle and serum was made by the method of Sunderman and Williams.[2] In order to obtain sufficient material for analysis the muscles from several animals in the younger groups were pooled.

The average values and standard errors for the analyses and calculations are given in Table I. It is to be noted that the approach of maturity is associated with a decreased concentration of chloride

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[1] Hastings, A. B., and Eichelberger, L., *J. Biol. Chem.*, 1937, **117**, 73.

[2] Sunderman, F. W., and Williams, P., *J. Biol. Chem.*, 1933, **102**, 279.

TABLE I.
Analyses of Rat Muscle and Serum.

Age in days	No. of rats	Per 100 g wet muscle			Per 100 g serum		G per 100 g		% H_2O in muscle cells
		% H_2O	mM Chloride	g fat	% H_2O	mM Chloride	Muscle cell phase	Non-muscle cell phase	
15	20	79.69 ±.14	3.171 ±.023	2.41	93.77	9.416	60.21 ±.83	39.79 ±.83	80.02 ±.23
30	23	77.87 ±.16	2.059 ±.038	2.06	93.39	10.415	76.48 ±.33	23.52 ±.33	79.56 ±.18
60	22	76.62 ±.06	1.588 ±.023	1.25	92.79	10.338	81.81 ±.24	18.19 ±.24	77.09 ±.07
90	16	75.88 ±.11	1.320 ±.021	0.69	92.23	10.158	85.14 ±.22	14.86 ±.22	75.80 ±.15
120	15	75.95 ±.16	1.413 ±.037	0.50	92.49	10.525	84.77 ±.38	15.23 ±.38	75.69 ±.16
180	13	75.72 ±.10	1.309 ±.029	0.67	91.58	10.116	85.33 ±.31	14.67 ±.31	75.61 ±.21
365	13	75.59 ±.14	1.410 ±.074	1.08	92.03	10.087	83.62 ±.79	16.38 ±.79	75.77 ±.45

and water in muscle and serum. This change in muscle composition was pointed out by Yannet and Darrow[3] in a study upon the tissues of young and old cats, the exact ages of which were not known. No appreciable change in chloride and water concentration occurred after 90 days of age. The neutral fat concentration in the muscles tended to follow somewhat the changes in chloride during the growth period but was found to be increased in the muscles of the older animals.

The relative mass of the non-muscle cell phase (connective tissue) decreases with age and reaches a value which is characteristic of the adult when the animals are somewhere between 60 and 90 days of age. The calculated values for the relative mass of muscle cells in the total tissue show a range of 60% at 15 days to 85% at 90 days of age. These differences are of sufficient magnitude to warrant consideration in any quantitative analysis of muscle power exhibited by muscle from animals of different ages. It is apparent that muscle growth is associated with a relative increase in the mass of chloride-free muscle cells and a reduction in the mass of connective tissue.

The lack of information concerning the true water concentration in connective tissue at the various ages prevents an accurate estimation of the concentration of water in the muscle cells. The values given in Table I should be considered as approximations only. However, when any reasonable assumption as to the amount of water in connective tissue is made it appears that the muscle cells from young rats have a higher concentration of water than cells from adult animals.

Summary. Determinations have been made of the concentration of chloride, fat and water in the gastrocnemius muscles and serum from 7 groups of rats ranging from 15 to 365 days of age. From these data calculations have been made concerning the relative masses of connective tissue and muscle cell phases present in the muscles at the various ages. Values characteristic of adult tissue are found at 90 days of age. Thereafter approximately a constant composition was observed. The calculated values for the connective tissue phase show a range of 40% of the total mass at 15 days to 15% at 90 days of age. The calculated percent of water in muscle cells decreases during the growth period.

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[3] Yannet, H., and Darrow, D. C., *J. Biol. Chem.*, 1938, **123**, 295.

10824

Penetration of Antiserum into the Central Nervous System of Monkeys Infected with Poliomyelitis.* II.

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It has been shown in the monkey infected with poliomyelitis that foreign serum injected intravenously cannot be detected in significant amounts in the cerebrospinal fluid nor in the CNS tissue.[1, 2, 3] Other investigators,[4-7] using a variety of antibodies in normal and diseased conditions have noted similar impermeability of the blood-brain barrier.

Netter and Debré[8] in 10 cases of meningitis noted the presence of horse serum in the blood of 8 patients less than 20 minutes after intrathecal injection. Stern and Gautier[5] introduced dyes and antiserum into the spinal fluid and readily detected them in the blood, while the same substances when given intravenously did not appear in significant quantities in the cerebrospinal fluid. McKinley and Holden[9] found *B. coli* "D" phage filterable from the cerebrospinal fluid into the blood, but not in the reverse direction. Burtenshaw[4] following intraspinal injection of horse antimeningococcus serum observed that it rapidly decreased in quantity in the spinal fluid and quickly appeared in the blood. Within one or two days the cerebrospinal fluid content fell to the low levels attained by parenteral administration alone.

Friedemann, Zuger and Hollander[10] believed that the cerebral capillaries of the guinea pig and rabbit are permeable to antibodies, because in their experiments tetanus antitoxin given intravenously

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* This work was aided by a grant from the Clara Ward Seabury Clinic for Infantile Paralysis.

[1] Shaughnessy, H. S., Grubb, T. C., and Harmon, P. H., *J. Bact.*, 1937, **33**, 59.

[2] Lennette, E. H., and Campbell, D. H., PROC. SOC. EXP. BIOL. AND MED., 1939, **41**, 320.

[3] Kempf, J. E., Nungester, W. J., and Soule, M. H., PROC. SOC. EXP. BIOL. AND MED., 1939, **40**, 395.

[4] Burtenshaw, J. M. L., *Lancet*, 1938, **2**, 1513.

[5] Stern, L., and Gautier, R., *Arch. Intern. Physiol.*, 1921, **17**, 138.

[6] Katzenelbogen, S., and Goldsmith, H., *Am. J. Psychol.*, 1931, **10**, 1045.

[7] Hektoen, L., and Carlson, J., *J. Infect. Dis.*, 1910, **7**, 319.

[8] Netter, A., and Debré, R., *Comp. Rend. Soc. de Biol.*, 1909, **67**, 100.

[9] McKinley, E. B., and Holden, W., PROC. SOC. EXP. BIOL. AND MED., 1927, **24**, 595.

[10] Friedemann, U., Zuger, B., and Hollander, A., *J. Immunol.*, 1939, **36**, 219.

neutralized tetanus toxin injected directly into the ventricles. On the other hand, Ramon and Descombey[11] produced a fatal tetanus in guinea pigs with blood from rabbits which had a few hours previously received intraspinal tetanus toxin. It would seem that Friedemann's, Zuger's and Hollander's results may be explained by assuming that the toxin injected into the cerebrospinal fluid passed into the blood stream and was there neutralized by the circulating antibodies. Since tetanus symptoms did not develop in their untreated animals until approximately 7 days after inoculation, toxin was not in the nerve tissue in significant quantities but may have been circulating in the blood in accordance with the work of Abel.[12] Moreover, mechanical rupture of blood vessels and resultant inflammatory reactions following intracranial inoculation might afford another direct route for the passage of toxin into the blood.

It is widely believed that antiserum injected intrathecally penetrates the CNS tissue. However, Flexner and Lewis[13] found it difficult to neutralize poliomyelitis virus suspensions in amounts as small as 1/50 cc when they were administered intracranially and the antiserums were given intrathecally at the same time. Cestan, Riser, and Labordee,[14] on injecting ferrocyanide into the ventricles during life and examining for Prussian blue granules post mortem, detected none deep in the nerve tissue. On such a basis they concluded that the cerebrospinal fluid does not penetrate the nerve cell, and that the intrathecal method of therapy is of no avail.

In the present work an attempt was made to demonstrate the penetration into the CNS tissue of rabbit hemolysin serum against sheep red blood cells following cisternal injection in animals during the active stage of poliomyelitis. Monkeys previously inoculated intracranially with Aycock's strain of poliomyelitis virus were given cisternally one to 2 cc of rabbit hemolytic serum with a titer varying from 1:30,000 to 1:40,000. After a definite time interval had elapsed (Table I), the animals were sacrificed. Spinal puncture was then performed, and specimens of spinal fluid were withdrawn. Subsequently, various portions of the brain and spinal cord were removed, and approximately 0.5 g samples mixed with physiological saline solution and ground with sand. The resulting suspensions were centrifuged and the supernatant fluid was tested for hemolysins according to the technic described in a previous paper.[3]

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[11] Ramon, G., and Descombey, P., *Comp. Rend. Soc. de Biol.*, 1931, **108**, 358.

[12] Abel, J. J., *Science*, 1934, **79**, 63.

[13] Flexner, S., and Lewis, P. M., *J. A. M. A.*, 1910, **54**, 178.

[14] Cestan, Riser, Labordee, *Medicine*, Paris, 1926, **7**, 1931.

TABLE I.

Penetration of Hemolysin in Nerve Tissue of Monkeys with Poliomyelitis.

Monkey	Stage of disease when hemolysin was injected	Hr between injection and sacrifice	Titer of hemolysin in blood	Titer of hemolysin shown as % of corresponding blood sample*					
				Spinal fluid	Lumbar cord	Cervical cord	Medulla	Site of inoculation of lesion	Frontal lobe
6	Quadriplegia	12	1:320	100	0	0	0	0	0
7	,,	6	1:640	6.2	0	0	0	12	0
8	,,	7	1:640	0	0	0	0	0	0
9	,,	10	1:1280	50	0	0	0	0	0
10	,,	12	1:2560	3	1.5	1.5	3	1.5	1.5

*The lowest dilution of tissue not anti-complementary was 1:40.

In a period of 12 hours or less (Table I) hemolysin could be detected in the blood in a minimum titer of 1:640 in 4 out of 5 cases. A titer as low as 1:320 was noted in only one instance. The hemolytic titers in the cerebrospinal fluid were definitely lower than the titers of the blood. The CNS tissues in most instances showed no hemolysin, the lowest dilution examined being 1:40. In one sample of brain taken from the site of inoculation of the virus, the hemolysin titer was 12% of the blood value.

These findings demonstrate that practically none of the hemolysin administered into the cerebrospinal fluid in monkeys infected with poliomyelitis actually reaches the CNS. The antibodies introduced left the cerebrospinal fluid within the experimental time of 6 to 12 hours and were demonstrable in high titer in the circulating blood.

Shaughnessy[15] has made similar observations.

10825

Effects of Activity upon Tissues of the Rat.

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This investigation was undertaken in order to determine the effect of different degrees of activity upon the weight and composition of tissues of growing animals. It was thought that this information might throw some light upon the mode of adjustment of the organism to the effects of chronic exercise and aid in the interpretation of observations made upon animals in which muscular activity was a variable factor. In this report are included observations of body and heart weights and the concentration of creatine, hemoglobin, total nitrogen and water in the skeletal muscle of 2 groups of albino rats. Litter mates after being weaned at the age of 5 weeks were equally distributed between 2 groups. The activity of one group, designated as inactive, was restricted by confining the animals to small individual cages for a period of 6½ months. The cages measured 21 cm in diameter by 22.5 cm in height and were almost completely filled with cut paper to further restrict movement. The other group, referred to as active, were housed in revolving activity cages.[1] Food and water were allowed *ad libitum*. The animals were

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[15] Shaughnessy, H. J., personal communication.

[1] Durrant, E. P., *Am. J. Physiol.*, 1924, **70**, 344.

TABLE I.

Condition	No. in group	Age in days	Dried heart wt / Live body wt	Avg daily run kilometers	Skeletal muscle			
					H_2O %	% Nitrogen	Mg Hb per 100 g	Mg creatine per 100 g
Active female P.E.M.	12	220	.090 ±.001	3.62 ±0.30	75.63	13.89	185 ±8	498 ±4
Inactive female P.E.M	12	226	.081 ±.001		75.61	13.84	152 ±3	497 ±4
Active male P.E.M.	11	227	.078 ±.001	2.79 ±0.25	76.11	14.02	192 ±7	527 ±6
Inactive male P.E.M.	13		.076 ±.001		76.03	13.98	161 ±7	505 ±5
Active male and female P.E.M.	23	222	.084 ±.001	3.29 ±0.17	75.87	13.96	188 ±5	510 ±4
Inactive male and female P.E.M.	25	225	.078 ±.001		75.82	13.91	156 ±3	501 ±2

killed by bleeding from the cut liver. During the hemorrhage an infusion of saline solution was made into a femoral vein according to the technic described by Whipple,[2] in order to wash the tissues free from blood. The muscles of the unoperated limb were freed as far as possible from fat and connective tissue, clipped into fine bits and aliquots were taken for analysis. Muscle hemoglobin was estimated according to the method of Whipple.[2] The acid hematin standard was employed. Creatine was determined according to Rose, Helmer and Chanutin.[3] The excised heart was opened, compressed against blotting paper, weighed and then dried in an electric oven to constant weight.

There was no significant difference between the body weights of active and inactive female rats. The mean body weight of the inactive males was 13% greater than that of the active group. The water content of the skeletal muscle was essentially the same in all groups. No significant difference was noted in the nitrogen content of skeletal muscle from the active and inactive groups. The creatine concentration in the skeletal muscle was the same in both female groups. The slightly higher mean values for muscle creatine in the active group of males is not regarded as significant. The extractable muscle pigment expressed as hemoglobin was consistently higher in the active animals than in those reared under restricted conditions. It is apparent from the data concerning the ratio of dried heart weight to total body weight that the relative heart weights of the active females exceeded that for the inactive females by approximately 10%. The difference in heart size between the active and inactive males was not significant. In this connection it is to be noted that the activity of the female group as judged by cage revolutions, exceeded that of the male group by 23%. Moreover, the intensity of the exercise may have differed greatly in the two sexes.

The differences in the activity of the active and inactive groups are greater than that permitted in animals reared under the usual laboratory conditions. In view of our findings it is permissible to conclude that any differences that might be found in the creatine, total nitrogen and water content of muscle from animals reared under ordinary laboratory conditions could not be due to differences in activity. It is evident that more intensive exercise than that experienced in spontaneous activity is required in order to bring about significant differences between the concentration of these constituents in active and inactive animals. However, differences in activity comparable to those in the above groups are accompanied by significant differences in muscle hemoglobin. This is in accord with the observation of Whipple[2] upon the differences in concentration of muscle pigment in dogs of different breeds and habits.

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[2] Whipple, G. H., *Am. J. Physiol.*, 1926, **76**, 693.

[3] Rose, W. C., Helmer, O. H., and Chanutin, A., *J. Biol. Chem.*, 1927, **75**, 543.

10826

Attempts to Demonstrate Vasopressor Properties in the Serum of Hypertensive Dogs.

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Several workers have reported that partial occlusion of the renal artery leads to the accumulation of a pressor substance in the ischemic kidney which may be demonstrated in suitable prepared extracts of the organ.[1-4] The inference that this substance (renin) is liberated into the circulation with consequent peripheral vasoconstricting action has led to attempts to show that the blood of subjects with ischemic kidneys and hypertension has excessive pressor properties compared to blood from normal subjects. Various test objects have been employed in 2 general methods of approach, (a) injection or perfusion of extracts of blood of hypertensive subjects, and (b) injection, perfusion or cross-circulation of whole blood or serum from hypertensive subjects. By the latter and most significant approach, contradictory results have been obtained.[5-12]

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[1] Harrison, T. R., Blalock, A., and Mason, M. F., PROC. SOC. EXP. BIOL. AND MED., 1936, **35**, 38.

[2] Harrison, T. R., Blalock, A., Mason, M. F., and Williams, J. R., Jr., *Arch. Int. Med.*, 1937, **60**, 1058.

[3] Prinzmetal, M., and Friedman, B., PROC. SOC. EXP. BIOL. AND MED., 1936, **35**, 122.

[4] Govaerts, P., and Dicker, E., *Compt. rend. Soc. biol.*, 1936, **122**, 809.

[5] Prinzmetal M., Friedman, B., and Rosenthal, N., PROC. SOC. EXP. BIOL. AND MED., 1936, **34**, 545.

[6] Prinzmetal, M., Friedman, B., and Rosenthal, N., PROC. SOC. EXP. BIOL. AND MED., 1936, **34**, 543.

[7] Collins, D. A., and Hoffbauer, F. W., PROC. SOC. EXP. BIOL. AND MED., 1937, **35**, 539.

[8] Dicker, E., *Compt. rend. Soc. biol.*, 1936, **122**, 476.

Recently Fasciolo, Houssay and Taquini[12] have described a method by which they state that blood taken from the renal vein of an ischemic dog kidney was shown to have increased vasoconstricting properties compared to blood which has passed through a normal kidney. They perfused a Läwen-Trendelenberg preparation[13] of the South American toad (*Bufo arenarum* Hensel) with serum which had been diluted to 8 volumes with Ringer solution, and which contained a final concentration of 0.5% sodium citrate. The blood was drawn directly from the renal veins of normal and hypertensive dogs under chloralose anesthesia. The hypertension had been produced by the procedure of Goldblatt, *et al.*[14] The perfusion rate diminished when the diluted sera of blood from the renal veins of hypertensive dogs was substituted for Ringer's solution to a much greater degree than when the diluted renal venous sera from normal dogs was employed. The vasoconstricting action would gradually disappear when Ringer was again perfused. We have attempted to confirm these important observations.

Experimental. The procedure employed differed from that of Fasciolo, Houssay and Taquini in that the test objects were frogs, bullfrogs and toads from the southern part of the U. S., and the blood for perfusion was withdrawn from the renal veins of unanesthetized dogs with explanted kidneys.[15] In addition the rate of perfusion in the frog was calculated by noting the time required for delivery of unit volumes of 5 or 10 cc of perfusate rather than by drop recording. In other respects the description of their technic was essentially followed. After 5 to 30 minutes perfusion with Ringer, "normal" diluted serum was substituted for 10 to 30 minutes followed by a short perfusion with Ringer, after which the "hypertensive" serum was substituted. This order was, of course, suitably varied.

The results of 26 experiments are summarized in Table I. There was no consistent difference in the effects of "normal" and "hypertensive" sera.

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[9] Heymans, C., and Bouckaert, J. J., PROC. SOC. EXP. BIOL. AND MED., 1938, **39**, 94.

[10] Houssay, B. A., and Taquini, A. C., *Rev. Soc. Argent. Biol.*, 1938, **14**, 5.

[11] Houssay, B. A., and Taquini, A. C., *Rev. Soc. Argent. Biol.*, 1938, **14**, 86

[12] Fasciolo, J. C., Houssay, B. A., and Taquini, A. C., *J. Physiol.*, 1938, **94**, 281.

[13] Abderbalden, *Handbuch der Biologischen Arbeits—Methoden*, Abt IV, Teil 7B, p. 1754.

[14] Goldblatt, H., Lynch, J., Hanzal, R. F., and Summerville, W. W., *J. Exp. Med.*, 1934, **59**, 347.

[15] Rhoads, C. P., *Am. J. Physiol.*, 1934, **109**, 324.

TABLE I.
Test Objects: Bullfrogs 16, Frogs 8, Toads 2.

Alterations in Perfusion Rate Upon Changing Perfusate from: (the numbers refer to the times a given response was observed)																	
Ringer to Normal Serum			Ringer to Hypertensive Serum			Normal to Hypertensive Serum			Hypertensive to Normal Serum			Hypertensive Serum to Ringer			Normal Serum to Ringer		
—	+	0	—	+	0	—	+	0	—	+	0	—	+	0	—	+	0
4	7	10	1	3	7	5	2	3	6	1	2	3	0	3	5	0	4

In a series of 15 additional experiments several variations in technic were introduced. These included, (a) substitution of mammalian Ringer for frog Ringer, (b) elimination of citrate by employing heparin as an anti-coagulant, (c) addition of chloralose to the perfusion fluids and (d) equilibration of the perfusion fluids with O_2—5% CO_2 gas mixture. The results were not significantly different from those shown in Table I.

In our experience the Läwen-Trendelenberg preparation was not a very satisfactory means of studying the vascular effects of dog sera when southern frogs, bullfrogs and toads were employed as test objects. Large spontaneous variations in perfusion rate were encountered with Ringer's solution alone, the rate, on the whole, tending to gradually fall. Substitution of the diluted sera brought about either an increase, decrease or no change of rate. Irrespective of which sera were employed, the rate, after a variable period, would rapidly decrease, the extremities of the preparation would become edematous, and the flow would cease. Return to Ringer except in the early stages would not restore the original rate. The effects recorded in Table I are those occurring during the first part of each experiment before the alterations in rate had become irreversible.

From these experiments we conclude that no particlar pressor properties may be demonstrated in the renal venous serum of dogs with hypertension produced by partial constriction of the renal artery when the Läwen-Trendelenberg perfusion technic is employed with southern frogs, bullfrogs, and toads as test objects. The perfusion rate initially may be either increased or decreased by both "normal" and "hypertensive" serum. With prolonged perfusion both types of sera usually cause a profound decline or cessation of flow.

It is to be emphasized that the experiments of Fasciolo, Houssay and Taquini were performed with South American toads whose vascular responses may be different.

Summary. Particular pressor properties could not be demonstrated in serum of the renal venous blood of hypertensive dogs by use of the Läwen-Trendelenberg perfusion technic with bullfrogs, frogs, and toads as test objects.

10827

Proteolytic Enzymes and the Question of Protein Storage.*

U. S. Ashworth. (Introduced by Paul L. Day.)

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Many observers have noticed that the nitrogen excretion of an animal on a N-free diet will decline for long periods of time. This fact is difficult to explain on the basis of the depletion of a supply of "reserve" or "deposit" protein. The amounts of nitrogen excreted may account for a much larger storage of protein in the body than anyone has ever been able to demonstrate. That the decline in the rate of excretion of nitrogen may be attributed to a change in the mechanism for the catabolism of protein was suggested by the author several years ago.[1] The assumption is made that an animal with an enzyme system elaborated to catabolize excess dietary protein, which may very likely first be converted to tissue protein, will go on tearing down tissue protein when the supply of dietary protein is suddenly removed. Gradually, however, as the animal continues to live on a diet free of protein, the enzyme system will deteriorate and the catabolism of protein will decrease.

This contention is borne out by the data of Table I. These data show the arginase content of the livers of rats after living on a N-free diet more than 30 days, as compared with normal controls taken from a stock diet.

The method for arginase assay was a modification of that described by Lightbody.[2] A 30-minute digestion period at 37° and pH 9.4 was followed by aeration of the ammonia liberated at pH 5.0

TABLE I.

Rat pair No. and sex Days on the N-free diet	29F 35	30M 35	32M 33	33M 34	34F 34
Body wt of N-free rat:					
Beginning, g	258	216	114	110	170
Final, g	180	185	70	72	133
Body wt of control	285	415	220	76	163
Arginase activity*					
N-free rat	0.14	0.13	0.12	0.08	0.14
Control rat	0.25	0.24	0.26	0.24	0.25

*Arginase activity is expressed as mg of urea-N liberated per mg of fresh liver tissue during a digestion period of 30 min at 37°.

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* Research Paper No. 521, Journal Series, University of Arkansas.

[1] Ashworth, U. S., and Brody, S., *Missouri Res. Bull.*, 1933, No. 190.

[2] Lightbody, H. D., *J. Biol. Chem.*, 1938, **124**, 169.

by glycerol urease. Manganous sulfate was used as an activator. Every precaution was taken to treat identically the samples taken from the experimental and control livers. Of course the N-free animals lost considerable body weight while subsisting on the N-free diet. There was some question of whether to use controls weighing the same as the original weight of the experimental animals or the same as their final weight. However, when the arginase activity is calculated per unit weight of fresh liver it seems to matter little which weight control animals are used. The ages of N-free rats Nos. 32M and 33M were 68 days when they were killed. The control for 32M was also 68 days old (same litter) while the control for 33M was 27 days old but had about the same body weight. In each case there is considerably more arginase activity in the livers of the control animals.

Supplementary evidence is found in the fact that the proteolytic enzymes of the pancreas possess less activity after a month of nitrogen starvation. Assay was conducted by means of formol titration. Casein was the substrate. The slope of the titration curve was found by plotting the logarithms of the amounts of alkali used against the time of digestion at 37°. The average slope, measured in arbitrary units, was 26 ± 3 for 11 N-free rats as compared with 43 ± 7 for their 11 controls.

The conclusion is reached then that the arginase activity of the liver, the tryptic activity of the pancreas and presumably the activity of the other proteolytic enzymes of the body may be gradually lowered by the removal of dietary protein. This would account for the continued drop in the endogenous nitrogen excretion during long periods of nitrogen starvation, and would be in agreement with the hypothesis that there is no strictly minimum level of nitrogen excretion.

10828

Absorption of Water by Liver Slices from "Physiological" Saline Solutions.

WARREN M. SPERRY AND FLORENCE C. BRAND.

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In a recent publication Field, Belding, and Martin[1] concluded that "the variability of the Qo_2 (as determined on tissue slices), based upon wet weight determinations, is no greater than when based upon determination of dry weight." They concluded further that "organs and tissues differ significantly in respect to the mean value of wet/dry weight ratio" and suggested that "expression of Qo_2 as a function of wet weight will often be the procedure of choice." The conditions under which the wet weight was determined were not stated.* It is the purpose of this note to call attention to a source of error which may accompany the calculation of quantitative metabolic data, obtained on tissue slices, on the basis of wet weight.

In a study of cholesterol distribution in the brain, slices were left in "physiological" saline solutions (0.9% NaCl or Tyrode solution) for varying intervals before weighing for analysis. Certain discrepancies in the results led us to suspect that considerable amounts of water had been taken up. To investigate the question it was necessary to devise a method by which tissues could be sliced without wetting. This was accomplished in the following way: A wooden box was constructed with a sloping glass top, glass front and back, and holes, covered with rubber flaps and large enough to admit the hands, at each side. The dimensions were 19½" wide, 15" deep, 12½" high in front, and 8¾" high in back. The atmosphere within the box was kept saturated, or nearly so, with moisture by heating water in a metal cup or beaker by means of a steam coil. An automobile windshield wiper, working on the front window, made it possible to see into the box. Satisfactory conditions were indicated by heavy steaming of the glass windows, such that a visible film of moisture formed on the window within 5 seconds after stopping the wiper. This situation was obtained if the temperature of the water was kept at 60° to 80°, with the temperature of the air within the box at about 40°. No attempt was made at an exact definition of the conditions, which of course varied from day to day with fluctuations in room temperature and atmospheric humidity. The criterion of steaming was found to be adequate for satisfactory results.

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[1] Field, J., 2d, Belding, H. S., and Martin, A. W., PROC. SOC. EXP. BIOL. AND MED., 1939, **40**, 565.

*In a personal communication Dr. Field states that excised organs were placed in Ringer's solution at once. The tissue was then sliced with a blade kept moist with Ringer's. The time between the placing of the organ in Ringer's and the removal of tissue slices from Ringer's ranged from 3 to 5 minutes; extraneous moisture was then removed by turning the slices over and over on quantitative filter paper, slightly moistened with Ringer's (to avoid the possibility of removal of water from cells by dry paper), and the slices were weighed. Dry weights were determined at the end of the respiration measurements.

Livers were removed from rats immediately after killing and transferred to a porcelain plate covered with a paper towel within the box. They could be sliced in the moist atmosphere with a dry razor blade almost as readily as with a wet blade according to the customary procedure. The slices were either immersed in saline solution (0.9% NaCl, Tyrode, or Krebs) at room temperature in small beakers, or, in control determinations, transferred directly to small tared aluminum pans, made by bending pieces of thin sheet aluminum about 1 cm square, and weighed immediately. The slices were removed from the solutions after varying intervals and turned over with a spatula on hardened filter paper until the paper showed no evidence of wetting. They were then transferred to aluminum pans and weighed. The samples were dried over night in an oven at about 110° and weighed after cooling in a desiccator.

Under the conditions described no significant variation in the water content of the tissue occurs over long periods of time. The results of 3 experiments, typical of the 9 carried out, are shown in Table I. In one of the experiments the liver remained in the box for as long as 150 minutes without change. Some slices were transferred to aluminum pans and left within the box for from 13 to 43 minutes

TABLE I.

Constancy of Water Content of Liver Kept in a Moist Atmosphere.

Exp. 3		5		6	
Time* min	Water %	Time* min	Water %	Time* min	Water %
6	69.0	0	68.6	0	69.4
7	69.8	2	69.0	1	69.1
25	69.2	22	70.7	2	69.5
33	68.9	27	70.4	15	69.0
44	69.5	42	69.7	27	68.7
51	69.6	54	69.7	49	69.7
61	69.9	69	69.6	71	68.4
73	69.6				
85	69.6				

* From placing liver in box.

before weighing. The moisture content did not vary significantly from control determinations in which the tissue was weighed immediately after slicing.

The rapid uptake of water by liver slices immersed in saline solutions is demonstrated in Fig. 1 by curves obtained from a single liver. In order to bring out the magnitude of the effect the data are expressed as amount of water taken up per unit weight of dry tissue. The calculation is illustrated by the following example: Water content of liver (control) 69.5%, or 228 mg water per 100 mg dry tissue. Water content of liver after 10.5 minutes in 0.9% NaCl solution 77.5%, or 344 mg water per 100 mg dry tissue. The difference, 116 mg, represents the water taken up per 100 mg dry tissue.

It will be seen that in the experiment represented in Fig. 1 the slices absorbed an amount of water equal to their dry weight within less than 10 minutes and equal to twice their dry weight in about 1 hour. The rate of uptake was about the same for each of the solutions studied. Similar findings were obtained in a total of 7 experiments with sodium chloride and 3 each with Tyrode and Krebs solutions. There was considerable spreading of the curves, particularly above 10 minutes, but in most experiments the tissues took up an amount of water equal to their dry weight within 10 minutes.

It is well known[2] that whole isolated muscle swells in isotonic solution. The phenomenon has been studied in detail by Parry.[3]

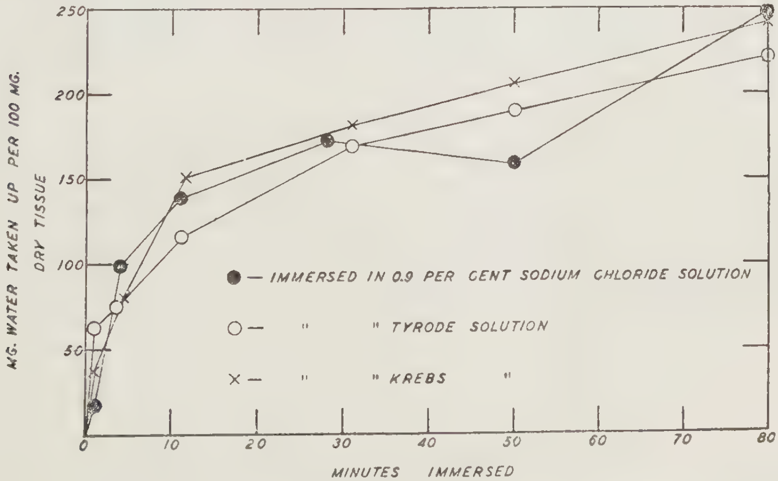

FIG. 1.

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[2] Adolph, E. F., *Am. J. Physiol.*, 1931, **96**, 598.

[3] Parry, A. A., *J. Cell. Comp. Physiol.*, 1936, **8**, 277.

The possibility that tissue slices would also take up water from isotonic solutions does not appear to have been generally recognized.

Adolph[2] showed that exchange of water by muscle occurred in proportion to the surface area and to the square root of the duration of immersion. Unfortunately it is not possible to calculate the surface area of the slices employed in our experiments. The average moist weight of the control samples was about 100 mg, but there was a wide variation from about 50 to 175 mg. Furthermore, although the great majority of the slices were within the range of thickness ordinarily employed in respiration experiments, no attempt was made to keep within close limits of tolerance. In a few instances exceptionally thick slices were noted to have taken up water at a low rate. Our primary purpose was to find conditions under which the uptake of water might be avoided; we were interested in establishing the magnitude of the effect more from the qualitative than the quantitative standpoint.

It is difficult to correlate the findings of Field, *et al.*,[1] with our results. In their experiments the tissues were immersed for about 3 to 5 minutes.* It is during this period that water is taken up most rapidly and, therefore, small differences in time should have introduced a considerable variation into their results. Furthermore, amounts of water such as they report for liver (ratio of wet to dry weight 5.07) were encountered in our experience only after 30 minutes to 1 hour in saline solutions. It is possible, though unlikely, that the rate of uptake from Ringer's solution is much faster than from the solutions employed in this investigation and that the maximum uptake had been approached by the time their slices were weighed.

Summary. Rat liver slices absorb water at a rapid rate from "physiological" saline solutions. An appreciable uptake may be observed within one minute; within 10 minutes an amount of water equal to the dry weight of the tissue is usually absorbed. Conditions under which tissues may be maintained for long periods of time without significant change in water content, and may be sliced without contact with solutions are described.

10829

Is Pantothenic Acid Essential for the Growth of Rats?

J. J. Oleson, D. W. Woolley and C. A. Elvehjem. (Introduced by E. B. Hart.)

From the College of Agriculture, University of Wisconsin, Madison.

The identification of the chick antidermatitis factor as pantothenic acid[1, 2] raises a question as to the need of this vitamin in the ration of rats. Lepkovsky, Jukes, and Krause[3] showed that rats required a factor in addition to riboflavin and vitamin B_6 and since it remained in the filtrate it was called the "filtrate factor." Their preparations were also active for chicks. Elvehjem, Koehn and Oleson[4] demonstrated the need for an additional factor for rats which was designated as factor W. Factor W was separated rather completely from the chick factor (pantothenic acid) but no evidence was presented to show that the rats required the chick factor in addition to factor W. Recently Subbarow and Hitchings[5] have obtained evidence that the active factor in their filtrate preparations is pantothenic acid. El Sadr, *et al.*,[6] have reported that their liver filtrate factor shows great resemblance to both pantothenic acid and factor W. Hoffer and Reichstein[7] have reported a growth response with β-alanine which they consider to be the active principle of pantothenic acid.

In our studies the following basal ration, which is similar to those used in our earlier work[8] has been used: sucrose 76%, purified casein 18%, salts III[9] 4%, corn oil 2%, supplemented with 1.2 mg thiamin*; 2.0 mg riboflavin, 2.5 mg synthetic vitamin B_6* (Merck),

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[1] Woolley, D. W., Waisman, H. A., and Elvehjem, C. A., *J. Am. Chem. Soc.*, 1939, **61**, 977.

[2] Jukes, T. H., *J. Am. Chem. Soc.*, 1939, **61**, 975.

[3] Lepkovsky, S., Jukes, T. H., and Krause, M. E., *J. Biol. Chem.*, 1936, **115**, 557.

[4] Elvehjem, C. A., Koehn, C. J., and Oleson, J. J., *J. Biol. Chem.*, 1936, **115**, 707.

[5] Subbarow, Y., and Hitchings, G. H., *J. Am. Chem. Soc.*, 1939, **61**, 1615.

[6] El Sadr, M. M., Hind, G. H., Macrae, T. F., Work, C. E., Lythgoe, B., and Todd, A. R., *Nature*, 1939, **144**, 73.

[7] Hoffer, M., and Reichstein, T., *Nature*, 1939, **144**, 72.

[8] Oleson, J. J., Bird, H. R., Elvehjem, C. A., and Hart, E. B., *J. Biol. Chem.*, 1939, **127**, 23.

[9] Arnold, A., and Elvehjem, C. A., *Am. J. Physiol.*, 1939, **126**, 289.

* We are indebted to Dr. D. F. Robertson of Merck and Co. for supplies of Thiamin, nicotinic acid, and vitamin B_6 and to Dr. C. Nielsen of Abbott Laboratories for supplies of haliver oil.

300 mg nicotinic acid,* and 300 mg choline per kilo. Each rat received 2 drops of haliver oil* per week.

A relatively crude preparation of pantothenic acid was used, which was prepared in the following way.† The liver extract was made alkaline and treated with norit. The filtrate and washings were acidified to pH 3 and again treated with norit. The factor was eluted from the second adsorbate with ammonia, readsorbed from acid solution and eluted again with a mixture of alcohol and pyridine. The eluate fraction was concentrated, $BaCO_3$ added to pH 8, and the mixture poured into a large volume of 95% alcohol. The precipitate was dissolved in water and reprecipitated and washed. The combined filtrates of the alcohol soluble Ba salts were concentrated to a thick syrup and thoroughly extracted with absolute alcohol. The combined filtrates were again concentrated to a syrup and the Ba salts were precipitated with acetone. These acetone insoluble Ba salts were freed from barium in the usual way. The final concentrate contained about 10% pantothenic acid as determined by bacterial assay.[10]

To inactivate the pantothenic acid, 1 cc of the above concentrate (containing about 70 mg of solids and 7 mg pantothenic acid), was added to 5 cc of normal NaOH and heated at 95° on the steam bath for one hour. The mixture was carefully neutralized and made up for feeding.

Rats placed on the basal ration at weaning failed to grow and plateaued at weights varying from 40 to 60 g. When 250 mg of liver extract was fed per day the growth rate was 3.4 g per day. A growth of only 2.4 g per day was obtained when the basal ration was supplemented with an amount of the crude preparation sufficient to supply 100 micrograms of pantothenic acid per rat per day. When the alkali-treated concentrate was fed no growth above that obtained with the controls was obtained. In another series of rats the average daily gain was 1.5 g when an amount equivalent to 50 micrograms per day was used and 1.7 g when the level was equivalent to 150 micrograms per day. Thus no significant improvement resulted when the intake was increased 3-fold. These records are calculated for a growth period of 6 weeks and if we study the weekly growth increments we find that the rats on the concentrate grew best the first 2 or 3 weeks and then showed a considerable decrease in the growth rate. Similarly a poorer response was obtained if the supplement was added after the rats had been on the basal ration for 3 weeks. Evidently the rats become depleted in factor W during this time.

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† A large proportion of the pantothenic acid was prepared by Dr. David Klein of Wilson Laboratories.

[10] Snell, E. E., Strong, F. M., and Peterson, W. H., *Biochem. J.*, 1937, **31**, 1789.

A small sample of crude crystalline material, made from the lactone of the hydroxy acid portion of the pantothenic acid molecule and reconjugated with β-alanine gave a growth of 1.1 g per day. The material was fed at a level equivalent to 50 micrograms of pantothenic acid per rat per day. Thus none of the concentrates gave growth equivalent to that obtained with liver extract, due to the lack of factor W, especially in the case of more purified preparations. Experiments on the supplementary action of pantothenic acid and factor W will be published later.

It is evident that the rat required in addition to the crystalline factors supplied in our basal ration an alkali labile factor, which is probably pantothenic acid. This factor will not completely replace the original liver extract or crude filtrate preparations, showing further the need for factor W.

The rat apparently requires the intact pantothenic acid molecule since the alkali-treated concentrates contain both the β-alanine and dihydroxy acid fractions. Stimulation due to β-alanine as present in our alkali-treated concentrates was not significant.

10830

The Metabolism of Human Spermatozoa.*

JOHN MACLEOD. (Introduced by J. C. Hinsey.)

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Though several papers appear in the literature describing some aspects of the metabolism of mammalian spermatozoa, little information is available on the metabolic behavior of *human* spermatozoa. McCarthy, *et al.*,[1] investigated glycolysis in human semen by measuring the decrease in sugar concentration at various intervals after incubation of the semen at 38°C. After 24 hours of incubation, they found that 75 to 90% of the sugar had disappeared but that, in some cases, the amount of lactic acid formed was not enough to account for the sugar used. They concluded that lactic acid is not

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* Aided by a grant from the National Committee for Maternal Health, Inc.

[1] McCarthy, J. F., Stepita, C. T., Johnston, W. B., and Killian, J. F., PROC. SOC. EXP. BIOL. AND MED., 1927, **25**, 54.

the end product of glycolysis in human semen. Goldblatt,[2] using essentially the same technic, came to similar conclusions. In neither case was an investigation made of the amount of sugar lost by oxidation to CO_2 and H_2O.

The work to be described here is part of a program designed to study in detail the metabolism of human spermatozoa and the enzyme systems responsible for the maintenance of metabolic function. The technic used was that of Warburg.[3] All results were obtained manometrically at 37.5°C.

Fresh specimens of spermatozoa, obtained from young, healthy individuals, were received in the laboratory within one hour after ejaculation. Since, in these manometric experiments, it was desirable to measure acid production in a non-retentive bicarbonate medium, the spermatozoa were centrifuged slowly out of the seminal fluid and resuspended in Ringer's solution containing 200 mg % glucose. For glycolysis measurements, bicarbonate was added to make a final concentration of 0.03M. For measurement of respiration, M/15 phosphate was substituted for the bicarbonate to make a pH of 7.4. Cell counts and calculations of motility were then made upon these final suspensions before measured amounts were added to the Warburg vessels. Nearly maximal motility is retained in Ringer's solution. For aerobic glycolysis, the vessels containing the spermatozoa were equilibrated at 37.5°C with a gas mixture containing 95% O_2-5% CO_2 for 15 minutes and for anaerobic glycolysis, a gas mixture containing 95% N_2-5% CO_2 was used. Respiration measurements were made in air and 100% O_2 but within these tensions of O_2, no difference was found. Measurements of respiration and glycolysis were made over a 3-hour period and the results below are the average gas consumption and production over that period expressed as mm^3 of O_2 and CO_2 per million cells per hour. Examination of motility was made at the end of the experiment.

It should be said at the outset that human spermatozoa in Ringer-phosphate show practically no respiration, so little indeed, that figures for respiration will not be presented here. However, the aerobic production of lactic acid as measured by CO_2 displaced from bicarbonate is exceedingly high relative to anaerobic glycolysis. In 80 experiments, the mean figures for aerobic and anaerobic glycolysis are:

$mm^3CO_2/10^6$cells/hour in N_2	$mm^3CO_2/10^6$cells/hour in O_2
0.100	0.080

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[2] Goldblatt, M. W., *Biochem. J.*, 1935, **29**, 1346.

[3] Warburg, D., *The Metabolism of Tumors*, 1930, Constable & Co., Ltd., London.

It will be seen that aerobic glycolysis is 80% of the anaerobic. Since, under careful experimental conditions, normal tissue shows high respiration and little or no aerobic glycolysis, human spermatozoa must be considered anomalous in that nearly all of the energy required for normal function is derived from glycolysis and *not* from *respiration.* This is demonstrated clearly in motility measurements made under aerobic and anaerobic conditions. Even after 10 hours in N_2, maximum motility is maintained and the glycolysis over that period, in most cases, remains linear. The same cannot be said of motility and glycolysis under aerobic conditions. Aerobic glycolysis after 3 hours tends to fall off with time and, after 3 or 4 hours, motility in O_2 shows a corresponding decrease. There are definite indications that the presence of O_2 inhibits the normal behavior of human spermatozoa.

Summary. The metabolism of human spermatozoa in Ringer-glucose is almost exclusively glycolytic and not respiratory. Aerobic lactic acid production is 80% of the anaerobic and falls off with time whereas anaerobic glycolysis is linear over a period of many hours. Maximal motility is maintained for many hours under anaerobic conditions but shows a marked tendency to decrease in air or in pure O_2. This suggests that the presence of O_2 has an inhibiting effect on the normal function of human spermatozoa.

10831

Relaxation of the Pelvic Ligaments of the Guinea Pig Induced by Progesterone.*

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From the Department of Physiology, Wayne University College of Medicine, Detroit, and the Department of Zoology, The State University of Iowa, Iowa City.

Early in the search for the active principle of the corpus luteum, 2 methods of extraction were reported to yield substances differing somewhat in their physiological effects. The crude extracts of Corner and Allen[1] induced progestational changes in the test rabbit.

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* This work was carried out at the Biological Laboratory, Cold Spring Harbor, L. I., N. Y.

† John D. Jones Scholar, the Biological Laboratory, 1939.

[1] Corner, G. W., and Allen, W. M., *Am. J. Physiol.*, 1929, **88**, 326.

Hisaw and his collaborators[2, 3] described an alcohol-insoluble fraction separable from corpus luteum extracts which possessed the specific capacity of causing relaxation of the guinea pig pubis—a phenomenon which normally occurs toward the end of pregnancy. This substance was given the name "relaxin" by these investigators, who considered it to be separate and distinct from the progestational fraction which they obtained in an ether-soluble form.

A hormonal control of the pelvic changes in the guinea pig, based upon an 'estrin-relaxin' action, has been described in detail by Hisaw,[4, 5] but it would appear that the need for postulating a hormone specific for the production of pelvic relaxation should not be considered imperative until the purified hormone of the corpus luteum, progesterone,[6, 7] has been demonstrated incapable of evoking this response. With the advent of the crystalline substance the possibility of putting it to test appears, singularly enough, to have escaped attention. Accordingly, experiments were devised to determine its adequacy in fulfilling, in addition to its well demonstrated activities, the one of causing relaxation of the pelvic ligaments in the virgin female guinea pig.

Adult females, weighing 350 to 634 g, were oöphorectomized under ether anesthesia and beginning 3 to 4 days later were injected with varying dosages of estrogens,‡ followed by a course of progesterone injections, either alone or together with continued estrogen administration. Dosages and details of administration are listed in Table I, which demonstrates that in all cases in which progesterone was given, in total quantities from 0.8 to 2.2 mg, marked relaxation occurred, easily detectable on palpation.

The last 3 experimental animals listed (Nos. 8, 9, 10), which received 3 daily injections of 1000 R.U. of estradiol benzoate, followed on the third day by a single injection of 1 mg progesterone and on the fourth day by 500 R.U. of estradiol benzoate and 0.2 mg progesterone, revealed definite relaxation, although not so pronounced

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[3] Fevold, H. L., Hisaw, F. L., and Meyer, R. K., PROC. SOC. EXP. BIOL. AND MED., 1930, **27**, 604.

[4] Hisaw, F. L., PROC. SOC. EXP. BIOL. AND MED., 1926, **23**, 661.

[5] Hisaw, F. L., *Physiol. Zool.*, 1929, **2**, 59.

[6] Fels, E., und Slotta, K. H., *Klin. Wochenschr.*, 1931, **10**, 1639.

[7] Allen, W. M., *J. Biol. Chem.*, 1932, **98**, 591.

‡ We are grateful to Dr. Oliver Kamm and Dr. D. A. McGinty of Parke, Davis & Company, for generous quantities of estrone (Theelin) and to Dr. Erwin Schwenk, of the Schering Corporation, for his kindness in supplying estradiol benzoate and progesterone (Progynon-B and Proluton).

TABLE I.
Summary of Cases of Pelvic Relaxation with Estrogen-progesterone Treatment.

Animal	Course of treatment	Results Relaxation of progesterone treatment
1	3000 R.U. estradiol benzoate per day for 4 days; 200 I.U. estrone plus 0.4 mg progesterone on 5th day; 0.4 mg progesterone on days 6 and 7	on 3rd day
2	3000 R.U. estradiol benzoate for 4 days; 200 I.U. estrone plus 0.4 mg progesterone on days 5, 6, 7	” 2nd ”
3	3000 R.U. estradiol benzoate for 4 days followed by 200 I.U. estrone plus 1.4 mg progesterone on days 6 and 7	” 3rd ”
4	3000 R.U. estradiol benzoate for 4 days; 200 I.U. estrone plus 0.6 mg progesterone on day 5, and 200 I.U. estrone plus 0.4 mg progesterone on days 6 and 7	” 3rd ”
5	3000 R.U. estradiol benzoate for 4 days; 250 R.U. estradiol benzoate plus 0.4 mg progesterone on days 5, 6, 7	” 4th ”
6	Same treatment as No. 5	” 1st ”
7	3000 R.U. estradiol benzoate on 1st 4 days; 250 R.U. estradiol benzoate plus 0.4 mg progesterone on days 5 and 7	” 1st ”
8	1000 R.U. estradiol benzoate for 2 days; 1000 R.U. estradiol benzoate plus 1 mg progesterone on 3rd day; 500 R.U. plus 0.2 mg progesterone on 4th day	” 2nd ”
9	Same treatment as No. 8	” 2nd ”
10	” ” ” ” 8	” 2nd ”
11	3000 R.U. estradiol benzoate on 1st 4 days; 250 R.U. estradiol benzoate on days 5, 6, 7	No relaxation
12	Same treatment as No. 11	” ”
13	1000 R.U. estradiol benzoate for 3 days; 500 R.U. on 4th day	” ”
14	Same treatment as No. 13	” ”
15	” ” ” ” 13	” ”

as in those animals receiving treatment over longer periods. The optimal combination of estrogen-progesterone has not been determined. Controls, receiving estrogen alone, as the table indicates, showed no relaxation. It would appear, therefore, that the crystalline hormone, progesterone, is quite effective, when administered to the oöphorectomized and estrin-primed guinea pig, in inducing relaxation of the pelvic ligaments.

10832

Lipid Distribution in Rat Liver After Partial Hepatectomy.

STEPHAN LUDEWIG, G. R. MINOR AND J. C. HORTENSTINE.
(Introduced by H. E. Jordan.)
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It is commonly observed that the liver remnant becomes fatty during the first 24 hours following partial hepatectomy.[1, 2] Following this period, gross examination indicates a disappearance of the fatty liver. The present communication is a study of the lipid constituents of the livers of rats following partial hepatectomy.

Inbred male rats, 60 to 70 days of age, of Wistar stock were used as experimental animals. Between 65 and 70% of the total liver was removed according to the procedure of Higgins and Anderson.[3] These animals were fed a stock diet (Bal Ra) and were allowed food up to the time they were killed.

Analyses for total fatty acids, phospholipids, free and total cholesterol and iodine numbers were begun immediately after the liver was removed under ether anesthesia. During the first few days after partial hepatectomy, the entire liver remnant was used for analysis. After appreciable hypertrophy had begun, wedges were removed from different portions of the liver for analysis. Total fatty acids were determined after saponification.[4] Cholesterol determinations were carried out according to the method of Schoenheimer and Sperry.[4, 5] The phospholipid fatty acids, obtained after saponification, were determined by the oxidation procedure of Bloor.[6] The iodine numbers of total and phospholipid fatty acids were done according to the procedure outlined by Yasuda.[7] Total solids were obtained after heating tissue for 48 hours at 105°C.

Total Fatty Acids. On the first day after partial hepatectomy the fatty acid concentration of the wet tissue increases from the mean control value of 3.5 to 8.4%. There is a marked decrease in con-

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[2] MacKay, E. M., and Carne, H. O., PROC. SOC. EXP. BIOL. AND MED., 1938, **38**, 131.

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[7] Yasuda, M., *J. Biol. Chem.*, 1931-32, **94**, 401.

TABLE I.
Average Liver Lipid Values of Partially Hepatectomized Rats.
Results are calculated on a dry fat-free weight basis in g per 100 g.

Days after operation	Neutral fat	Phospholipid	Total Cholesterol	Free Cholesterol	Esterified Cholesterol	Total Lipid	Phospholipid to neutral fat ratio
Controls	4.9	11.5	1.09	0.86	0.23	17.5	66:28
1	23.6 (380)*	17.5 (52)	1.68 (54)	1.10 (28)	0.58 (152)	42.8 (145)	41:55
2	15.8 (223)	14.3 (24)	1.60 (47)	1.15 (34)	0.45 (52)	31.7 (81)	45:50
3	7.2 (47)	13.1 (14)	1.31 (20)	1.09 (27)	0.22 (—4)	21.7 (24)	57:33
4	7.3 (49)	12.4 (8)	1.22 (12)	1.10 (28)	0.12 (—48)	20.9 (16)	59:35
7	5.5 (12)	12.3 (7)	1.13 (4)	0.97 (13)	0.16 (—30)	18.9 (8)	65:29

± Calculated on basis of total lipids.
* Values in parentheses represent percentage changes.

centration which approaches the control values on the third day. The mean iodine numbers of the fatty acids vary between 119 and 126 during the 7-day experimental period and statistical analysis shows no significant difference between any of the values obtained on different days.

Neutral Fat. The concentration of neutral fat in the dry fat-free liver is calculated from the available data (Table I). The greatest percentage increase of all the lipid constituents is recorded for the neutral fat during the first and second days after operation. On the seventh day the concentration approaches the control value.

Phospholipids. The phospholipid concentration in the dry fat-free liver (Table I) increases after operation but decreases slowly and does not reach the control level on the seventh day. There is a progressive increase in the iodine number from 125 to 159 during the experimental period.

Cholesterol. The concentration of free cholesterol in the dry fat-free liver (Table I) is increased slightly and remains elevated during the experimental period. The esterified cholesterol increases markedly during the first day and decreases below the control value on the third, fourth and seventh days.

Phospholipid-neutral Fat Ratio. Theis[8] has noted that the phospholipid-neutral fat ratio of the normal liver remains constant but changes markedly in the degenerating organ. These results have been confirmed, since the greatest deviation of this ratio from the normal occurs during the first 24 hours after operation. This change in ratio appears to be due to the proportionately large increase in the neutral fat concentration. After this time this ratio gradually increases and returns to the normal value on the seventh day.

Total Solids. The total solids of the dry fat-free liver decrease from the mean control value of 25.4% to 20.0 and 19.9%, respectively, on the first and second day after operation. There is a gradual increase on the third, fourth and seventh days to 21.0, 22.4, and 24.5%, respectively.

Brucs and coworkers[1] have shown that there is no mitotic activity in the liver remnant during the first 24 hours after partial hepatectomy. During this period the liver increases in size between 50 and 60%. Much of this increase in weight must be due to the increased lipid deposition since the water and glycogen[9] content decreases at this time. It is thus evident that cellular inactivity under these experimental conditions is accompanied by a marked deposition of lipids, particularly neutral fats. At the end of the first 48 hours, mitotic activity increases markedly and the liver remnant more than doubles its weight.[1] At this time the lipid concentration decreases appreciably but the water and glycogen are still low. Hence the increase in liver weight is probably due to new cells still containing appreciable amounts of lipid and a decreased water content. Further liver hypertrophy is associated with increased water and decreased lipid contents.

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[8] Theis, E. R., *J. Biol. Chem.*, 1929, **82**, 327.

[9] Stone, C. S., *Arch. Surg.*, 1935, **31**, 662.

The capacity of the liver to increase in size after partial hepatectomy can be compared with sarcomatous[10] and embryonic tissue.[11] The degree of unsaturation of phospholipid fatty acids of tumor tissue in the rat was found to be low by Haven.[12] In the regenerating liver the iodine number of the phospholipid fatty acids was found to be higher than normal. The amount of phospholipid in tumors of various types has been shown to be large by a number of workers[13] but the increase in the regenerating liver is small and not appreciable. Thus it can be seen that two rapidly growing tissues such as the liver and tumors show definite differences in their phospholipid content.

Summary. During the first 24 hours after partial hepatectomy, the liver remnant becomes fatty due particularly to the deposition of large amounts of neutral fat and to increases in the phospholipid and cholesterol fractions. On the second and third days there is an appreciably sharp decrease in the concentration of all the lipid constituents. After 7 days the lipid values are all slightly above the respective control values excepting the combined cholesterol which is decreased.

The iodine numbers of the total fatty acids remain constant but those of the phospholipids increase progressively during the experimental period.

Liver injury produced by partial hepatectomy results in a marked disturbance in lipid metabolism. Regeneration of this tissue is accompanied by a return to normal values.

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10833

Intestinal Secretion During Anoxia.

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It has been shown previously[1, 2] that anoxia may cause a distinct diminution in urine secretion and that secretion of acid by the stomach is also decreased by anoxia.[3] In this paper the effect of anoxic anoxia on the secretion of *Succus entericus* will be reported.

Pairs of dogs as nearly the same weight as possible were used. Five hours before the experiment they were fed a standard meal, according to body weight, of cooked hamburger to encourage intestinal secretion. Under nembutal anesthesia loops of jejunum, measured to be the same length in both dogs of a pair, were washed with normal saline, stripped lightly between the fingers to remove the washing fluid, tied off, and returned to the peritoneal cavity. Thirty milligrams per kilo body weight of peptone extract prepared according to the method described by Nasset and Pierce[4] were then administered intravenously to stimulate intestinal secretion. One of the dogs was put into a low pressure chamber, the other served as a control at atmospheric pressure. Twenty minutes after the injection of the peptone extract the animal was sacrificed, the jejunal loop removed, and the amount of secretion it contained was measured.

Sixteen experiments were performed at a partial pressure of 80 mm Hg (equivalent approximately to an altitude of 18,000 ft.), and 15 at a partial pressure of 53 mm Hg (28,000 ft.), making a total of 31 pairs of dogs.

Results. The average secretion in cc of *Succus entericus* per dog was as follows:

TABLE I.

	cc
Average of all controls	14.0
’’ at 80 mm Hg O_2 pressure	14.5
’’ ’’ 53 ’’ ’’ O_2 ’’	9.9

Anoxia obviously produced no noticeable change at 80 mm Hg

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[2] Van Liere, E. J., Parker, H. S., Crisler, G. R., and Hall, J. E., PROC. SOC. EXP. BIOL. AND MED., 1935, **33**, 479.

[3] Pickett, A. D., and Van Liere, E. J., *Am. J. Physiol.*, 1938, **123**, 163.

[4] Nasset, E. S., and Pierce, H. B., *Am. J. Physiol.*, 1935, **113**, 568.

oxygen pressure; at 53 mm a slight depression of secretion appears. This apparent diminution, however, is not statistically significant.

The intestinal glands are seen to be rather highly resistant to anoxia, probably indicating that they have a low energy requirement for secretion. The questionable depression of secretion at 53 mm Hg oxygen pressure is slight at best; the glands are still secreting quite well. Not all anesthetized dogs will survive this degree of anoxia for 20 minutes.

Summary. Intestinal secretion in dogs was found to be resistant to anoxia. At a partial pressure of oxygen of 80 mm Hg (approximate altitude of 18,000 ft.) there was no effect, and at 53 mm Hg (28,000 ft.) only a mild depression of secretion, which was not statistically significant. This indicates a low energy requirement for intestinal secretion.

10834

Effects of Vitamin C Deficiency and Diphtheria Toxin on Cellular Blood Constituents of Guinea Pig.*

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The literature contains many reports concerning the anemia associated with scurvy in both humans and experimental animals.[1-10] There is general agreement that a continued lack of vitamin C in the diet causes a progressive decrease in erythrocytes and hemoglobin, and that the anemia becomes severe only during the later stages of a deficiency. The cause of the anemia is not well understood, however.

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* Contribution No. 391 from the Department of Chemistry, University of Pittsburgh.

† Parke, Davis & Co. and Abbott Laboratories Research Fellow.

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[9] Malmberg, J., and Euler, H. v., *Naturwistn.*, 1936, **24**, 713.

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Considerable evidence[5] indicates that it is due to cytological changes in the bone marrow, resulting in a retarded production of erythrocytes, rather than to a specific deficiency of hemoglobin or an excessive destruction of erythrocytes.

There is apparent disagreement concerning the effects of scurvy on the leucocyte count of the blood. Using guinea pigs, von Euler and Malmberg,[8] and Shimada[7] have reported a decrease in leucocytes accompanying the depletion of vitamin C, but Liotta[3] and Aszodi[10] have reported an increase under similar conditions.

While studying the effects of vitamin C depletion[11] and diphtheria toxin injections[12] on the glucose tolerance of guinea pigs, we were afforded an opportunity of studying the parallel changes in cellular blood constituents, with the kind assistance of Miss Sarita Bailey, of the Children's Hospital.

Essentially, we observed that a decrease in hemoglobin and erythrocytes, and a slight increase in leucocytes accompanied the development of scurvy. In the experiments with toxin we observed that the level of vitamin C intake influenced the effects of the toxin, as measured by changes in the cellular blood picture.

The results supplement our finding[12] that the effects of diphtheria toxin on the glucose tolerance of guinea pigs are more severe when the vitamin intake has been low. Numerous reports have shown that the vitamin plays a rôle in decreasing the severity of infection.[13–17] The increased protection afforded by a high vitamin intake is due apparently to the general physiological benefits of a better state of nutrition of the animals, rather than to a direct chemical inactivation of the toxin by the vitamin.[18] We have observed acidic inactivation of the toxin *in vitro,* but neutral, buffered solutions of the vitamin failed to exert a significant effect upon dilute solutions of the toxin.

Experimental. Guinea pigs weighing approximately 300 g were fed the basal diet of Sherman and collaborators[19] supplemented with cod liver oil, yeast (2%) and a solution of crystalline vitamin C until they had reached 450 to 600 g in weight. The animals were all of

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TABLE I.

Effects of Vitamin C Deficiency and Diphtheria Toxin Injections on Cellular Constituents of Blood of Guinea Pigs. All determinations were made on the 20th or 21st experimental day, except for the 10 days depletion group. Toxin injections, 0.2 M.L.D. each, were made on the 10th and on alternate succeeding days (5 injections).

Group	No. of animals	Hemoglobin %, Sahli	Erythrocytes, millions	Leucocytes, thousands	Polymorphonuclear leucocytes	Lymphocytes	Monocytes	Eosinophiles	Basophiles	Nucleated red cells per 100 leucocytes
3 mg/day, no toxin	16	87 (80-96)*	4.4 (4.0-4.9)	7.8 (6.1-10.8)	45 (30-57)	50 (38-61)	1.5 (0-4)	3.0 (0-8)	0.7 (0-4)	
3 mg/day, and toxin injections	10	79 (69-88)	4.0 (3.6-4.2)	9.7 (6.2-15)	45 (36-60)	51 (36-61)	2.8 (0-5)	2.0 (0-8)		
0.5 mg/day, no toxin	9	77 (70-84)	3.9 (3.5-4.2)	8.4 (6-11.2)	45 (30-62)	49 (36-60)	3.0 (0-6)	2.2 (0-14)	.8 (0-2)	
0.5 mg/day, and toxin injections	7	63 (45-80)	3.3 (2.4-4.2)	18.6 (12-34)	64 (52-78)	30 (18-44)	4.0 (2-8)	2.3 (0-8)		5† (0-24)
10 days depletion, no toxin	25	82 (69-90)	4.1 (3.5-4.6)	8.4 (6.1-15.5)	45 (26-54)	46 (32-62)	4.4 (0-8)	3.0 (0-12)	.5 (0-2)	
20 days depletion, no toxin	10	58 (22-78)	3.0 (1.5-3.9)	9.4 (7.5-12.2)	46 (32-57)	46 (34-70)	5.7 (2-8)	1.2 (0-4)	.4 (0-2)	75‡ (0-600)
20 days depletion, and toxin	6	49 (29-65)	2.8 (1.6-3.5)	17.5 (6.9-25.2)	68 (43-79)	27 (18-42)	3.3 (1-7)	2.3 (0-8)		81‡ (17-300)

* Extreme values for individual animals are given in parentheses.

† Moderate and marked chromatophilia, anisocytosis and poikilocytosis.

‡ Marked chromatophilia, anisocytosis and poikilocytosis.

approximately the same age. It had been found advisable to use fairly large animals for the sugar tolerance tests, to facilitate blood sampling.

Toxin. The diphtheria toxin was carefully standardized on an M.L.D. basis (238 M.L.D. per cc) with pigs weighing 250 g. Toxin dilutions in sterile physiological saline were standardized so that 1 cc of solution contained 1 M.L.D. of toxin. Injections were made subcutaneously.

Blood samples. The erythrocyte, leucocyte and differential counts were made in the usual manner upon blood samples obtained from a marginal ear vein.

Vitamin C depletion. Table I shows the effect of vitamin C depletion alone on the cellular blood constituents of guinea pigs that had been receiving 3.0 mg of vitamin daily for 20 days. The control determinations were made a few days before starting the depletion. During the 10-day depletion period there was a slight decrease in erythrocytes and hemoglobin and a slight increase in leucocytes. Between the 10th and 21st days of depletion the anemia became severe, with a parallel decrease in both hemoglobin and red blood cells and a further slight increase in leucocytes. Nucleated red cells and abnormal cell forms appeared during the onset of severe scurvy. All the animals began to lose weight and show signs of scurvy between the 10th and 15th days of depletion.

Animals receiving 0.5 and 0 mg of vitamin C per day were given 5 injections of 0.2 M.L.D. of toxin each. Injections were made on the 10th experimental day and at 2-day intervals thereafter. The determinations were made on the 20th or 21st experimental day. The data for these animals compared with control animals receiving the same amount of vitamin but no toxin are also given in Table I. The group receiving toxin and 3.0 mg of vitamin daily showed a moderate reduction in hemoglobin and erythrocytes and a moderate increase in leucocytes. The groups receiving toxin and 0.5 or 0 mg of vitamin C showed a greater drop in hemoglobin and red blood cells and a larger increase in leucocytes than shown by the 3.0 mg group. The differential counts of the toxin-treated, low-vitamin groups showed an increase in polymorphonuclear leucocytes, a decrease in lymphocytes, and the presence of many nucleated red cells and abnormal cell forms.

It is evident that the animals receiving a higher intake (3.0 mg) of vitamin C were able to withstand the effects of the toxin to a greater degree. The distinctly sub-optimum intake of vitamin (0.5 mg) was sufficient, however, to permit nearly normal growth and freedom from external signs of scurvy.

The results of the present investigation supplement, and in a way, confirm, the earlier findings in our laboratory concerning the lowered resistance against diphtheria toxin that characterizes a low intake of vitamin C. Tooth structure[20] and glucose tolerance[12] have been considered in the earlier papers.

Conclusions. Accompanying the anemia of scurvy in guinea pigs there was a progressive decrease in both hemoglobin and erythrocytes and a slight increase in leucocytes.

Guinea pigs receiving 3.0 mg of vitamin C per day and given subcutaneous injections of diphtheria toxin showed less disturbance in their blood cell count than similarly injected animals receiving 0.5 mg or less of the vitamin per day. The changes in the low-vitamin, toxin-injected groups were characterized by chromatophilia, anisocytosis and poikilocytosis.

10835

Antihormone Effects in Pancreatic Diabetes.

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Collip and Anderson[1] observed that during the prolonged injection of anterior pituitary extract (thyrotropic) its effect upon the metabolic rate was not sustained. They also noted[1, 2] that the serum of refractory animals inhibited the response of normal animals to thyrotropic hormone. Similar inhibitory effects have been observed in the case of the gonadotropic hormone. The relation of such antagonistic principles to species has been further studied.[3, 4, 5]

Relatively few reports are available on the development of principles which antagonize the diabetogenic action of the anterior pituitary, although refractoriness has long been recognized. Collip[6] reported one dog which was injected with pituitary extract and depancreatized; its diabetes resembled that of the Houssay animal. Similar experiments have been performed by Campbell, Keenan and Best.[7]

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We have used depancreatized dogs to study the effect on the diabetes (a) of prolonged treatment with pituitary extract and (b) of the injection of the serum from a normal dog treated with anterior pituitary extract. The influence of these procedures on the excretion of glucose, nitrogen and acetone bodies of the depancreatized animals during fasting and the omission of insulin has been recorded. When these criteria manifest the full diabetic response it is regarded (since Houssay) as the result of pituitary activity, and we have consequently assumed that the amelioration of diabetes indicates an "inhibition of the diabetogenic principle(s)."

Methods and Results. A. Intraperitoneal injections of a crude saline anterior pituitary extract (beef) were administered daily, with few exceptions to a female mongrel dog. During the tenth and eleventh month of injections blood serum was obtained and injected intraperitoneally into 2 completely depancreatized dogs. The serum was administered to the diabetic dogs the day before and the first 2 days of fasting. The results are shown in Table I. The injection of the serum from the donor dog was accompanied by a marked decrease in glycosuria and a slight decrease in urine nitrogen from the control levels. The blood sugars during the fasting periods

TABLE I.
Fasting Metabolism of Various Depancreatized Dogs—3-day Periods.

Dog	Date*	Wt, kg*	Total serum injected, cc	Glucose, avg g/day	Urine Nitrogen, avg g/day	Ketones avg mg/day
Control Periods.						
A-2	10/6/38	4.50	0	12.8	3.8	28
A-5	11/24/38	10.25	0	18.9†	4.2†	0†
A-6	12/24/38	6.05	0	14.8	5.0	593
A-8	4/14/39	7.15	0	22.9	7.8	1301
During Injection of Serum from Normal Dog.						
A-6	1/4/39	5.45	84	25.7	5.6	136
A-7	3/3/39	7.20	87	17.1	7.6	90
During Injection of Serum from Dog Treated 10 Months with Ant. Pituitary Extract.						
A-2	11/4/38	4.35	70	2.1	2.6	17
A-5	12/6/38	9.35	84	3.7	3.2	0

* 1st day of period.
† Loss of some urine during this period.
Insulin omitted during all periods.

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[6] Collip, J. B., *Glandular Physiology and Therapy*, Chicago, 1935, p. 105.

[7] Campbell, J., Keenan, H. C., and Best, C. H., *Am. J. Physiol.*, 1939, **126**, 455.

were within the range 132-372 mg per 100 cc, which may be found in the hypophysectomized-depancreatized dog. Injections of serum from normal dogs were not associated with a decrease in the glycosuria or urine nitrogen excretion.

It may be noted that Dog A-2 died from insulin shock 9 days after receiving the serum from the donor dog. At this time it was receiving a daily dosage of 18 units of insulin and eating 100 g of pancreas, 400 g of beef and 60 g of sugar a day. Dog A-5, after the last injection of serum from the donor dog, showed a marked reduction in insulin requirement. Seventeen days after this last injection it excreted only 0.5 g of sugar per day during a 2-day fast without insulin. The dog's appetite then failed, and it was sacrificed. The donor dog died from acute distemper several weeks after completion of this portion of the experiment.

The autopsies on the diabetic dogs as well as their fasting control periods indicated that the pancreatectomies were complete.

Autopsies. Dog A-2, November 13, 1938; wt. 3.55 kg. No pancreatic tissue was found. There was very little body fat. The liver was not grossly fatty. Microscopic sections of the pituitary, thyroid, ovary, adrenal and liver failed to reveal anything unusual.

Dog A-5, December 30, 1938; wt. 7.5 kg. No pancreatic tissue was found. There was ample abdominal fat. Liver glycogen 1.87%. Microscopic sections, as in Dog A-2, failed to reveal any definite abnormality.

B. It was the purpose of these experiments to determine the effect of prolonged intraperitoneal anterior pituitary extract injections in 2 completely depancreatized dogs. With rare exceptions they were maintained on a constant diet and insulin was given. The effect of cessation of anterior pituitary extract injections and insulin upon the fasting metabolism of these dogs is shown in Table II. There was a marked reduction in the fasting glycosuria and a slight decrease in nitrogen excretion when compared to the control periods. The blood sugars of dog A-8 during the 3-day fasting period starting 6/23/39 ranged from 158 to 168, and those of dog A-6 during the period starting 3/23/39 from 228 to 99 mg per 100 cc.

During the course of the daily injection of 2 cc of anterior pituitary extract into Dog A-8 there was first a marked diabetogenic effect so that the insulin was raised from 30 to 70 units. However, by the 15th day the insulin requirement was slightly less than before starting the extract. Dog A-6 also showed an increased and then a decreased diabetic response to anterior pituitary during the injections as measured by the insulin requirement.

TABLE II.
Effect of Prolonged Anterior Pituitary Extract Injection on the 3-day Fasting Metabolism of Depancreatized Dogs.

Date*	wt, kg	Period of injection ant. pituitary extract, days	Time† from last ant. pit. injection, days	Glucose, avg g/day	Urine nitrogen, avg g/day	Ketones, avg mg/day
No. A-6; Female; Pancreatectomy December 19, 1938.						
12/24/38	6.05	control	—	14.8	5.0	593
3/5/39	5.32	30	1	7.0	7.1	34
3/23/39	5.25	48	2	1.7	3.8	0
No. A-8; Female; Pancreatectomy April 10, 1939.						
4/14/39	7.15	control	—	22.9	7.8	1301
4/27/39	6.70	control	—	27.3	8.0	3267
6/23/39	6.35	38	4	0	3.7	36

* 1st day of period.
† To 1st day of period.
Insulin omitted during all periods.

After the final fasting periods, both dogs were fed but insulin was withheld. One showed a moderate glycosuria when fed in spite of the low sugar excretion during fasting. This implies that the fasting glycosuria was due to an inhibition of the production of glucose and not to its normal utilization. The other had only a slight glycosuria on feeding. Both dogs resembled the Houssay animal.

The autopsies as well as the control periods indicated that the pancreatectomies were complete.

Autopsies. Dog A-6, April 5, 1939; wt. 4.40 kg. Died in insulin shock. No pancreatic tissue found. Three small cysts were noted in the intermediary lobe of the pituitary; ample anterior lobe tissue of normal appearance was present. The thyroid gland was composed of thick epithelial cells and there was less colloid than usual. The appearance suggested thyroid stimulation of moderate degree. Adrenals, ovaries and liver were not remarkable; liver fatty acids were 1.83%.

Dog A-8, June 28, 1939; wt. 5.70 kg. No pancreatic tissue found. Small fat depots. The liver appeared grossly normal and microscopic examination showed some vacuolation; liver fatty acids were 4.94%; glycogen 0.37%. The thyroid showed slight evidence of stimulation; adrenals and ovaries not remarkable although ovarian follicles were large.

Sera from both dogs were kindly tested by D. J. Ingle for antidiabetogenic effect in fed, partially depancreatized rats. Due to changes in food intake the results were inconclusive, but certainly there was no marked effect such as that obtained by Long, Thompson and Fry[8] with their antiserum from a normal dog.

In addition, 5 cats were treated with pituitary extract for 2 months and were depancreatized a week after the last injection. For the first 3 days after operation the average glucose excretion was 1.75 g per kg per day, in contrast to 3.2 g per kg per day excreted by 16 untreated diabetic cats. The urine of all the injected cats was negative for acetone.

Discussion. Two depancreatized dogs given serum from a donor treated with pituitary extract had a diminished excretion of glucose and nitrogen. This did not occur when the serum of a normal dog was used. It appears that the passive transfer of a principle antagonistic to the diabetogenic hormone(s) of the anterior pituitary is demonstrated. This is comparable to the results obtained by others in the case of the thyrotropic and gonadotropic hormones. Since the action of the serum occurred during the absence of the islands functional changes in insulin secretion are eliminated and the "antifactor" thus appears to act on the pituitary or on the tissues.

A similar response occurred when the antifactor was actively induced by the administration of the extract to two depancreatized dogs. Here, too, any adaptation of the pancreatic islands such as might occur in an intact animal had been eliminated.

The cysts in the pituitary gland of Dog A-8 were probably not significant, since there was abundant anterior pituitary tissue present, and since there were no cysts in the pituitaries of the other dogs.

Conclusions. 1. Two completely depancreatized dogs given intraperitoneal injections of serum from a dog treated over 9 months with anterior pituitary extract showed a marked decrease in the degree of the glycosuria. (2) Two completely depancreatized dogs injected with anterior pituitary extract for 30 or more days showed, after cessation of extract injection, a much less severe diabetes during fasting than before the injections. (3) The metabolic behavior of these 4 dogs was much like that of the depancreatized-hypophysectomized animal.

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[8] Long, C. N. H., Thompson, K. W., and Fry, E. G., personal communication.

10836

Protective Value of Immune Rabbit Serum and Its Globulin Fraction Against Experimental Murine Infection with *Hemophilus pertussis*.*

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The intranasal inoculation of young mice with virulent *Hemophilus pertussis* produces an experimental disease against which the value of immunizing agents may be tested. By this method the protective capacity of hyperimmune human serum (lyophile) has been demonstrated.[1]

The present report describes the results obtained by the use of immune rabbit serum and of a globulin fraction prepared from it. Rabbits were immunized by the intravenous injection of suspensions of living *H. pertussis.* Three injections of 0.1, 0.3, and 0.5 cc of a standard suspension containing approximately 10 billion organisms per cc were given on successive days. After a rest period of a week, a second course was given. Trial bleedings were made 10 days after the last injection. When the agglutinating titer of the serum was 1:2560 or greater, the rabbits were bled from the heart, and the serum was separated and stored at 4°C.

The globulin fraction was prepared in the manner described by Heidelberger, Turner and Soo Hoo.[2] To 40 cc of serum were added 40 cc of sodium-sulfate solution, saturated at 37°C. The resulting precipitate was separated from the supernate by repeated centrifugation, and washed in sodium-sulfate solution, 60% saturated at 37°C. For the present experiments, it was deemed advisable to free the globulin from sodium sulfate, which was accordingly removed by dialysis against 0.85% sodium-chloride solution at 4-6°C. Asepsis was maintained throughout. The volume after dialysis was 26 cc. A small amount of insoluble matter was removed by centrifugation and the solution, now nearly clear and colorless, was diluted to 40 cc.

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* This investigation has been made with the assistance of a grant from the Committee on Therapeutic Research, Council on Pharmacy and Chemistry, American Medical Association.

†Working under a grant from the Fluid Research Fund of the University of Rochester.

[1] Bradford, W. L., and Wold, M., *Am. J. Dis. Child.*, in press.

[2] Heidelberger, M., Turner, J. C., and Soo Hoo, C. M., PROC. SOC. EXP. BIOL. AND MED., 1937, **37**, 734.

Tested against a suspension of *H. pertussis* containing 5 billion organisms per cc, the agglutinating titers of the various fractions were: Original immune rabbit serum, 1:5120; globulin fraction, 1:5120; supernate from original precipitation, 0; combined washings of precipitate, 1:10. The dilutions were directly comparable. It is obvious that, within the limits of accuracy of the determination of agglutinin, all of the agglutinating antibody was precipitated by 50% saturated sodium sulfate (37°C) and that none was lost in washing and dialyzing.

The nitrogen contents of the original serum and globulin fraction were respectively 10.6 and 3.6 mg per cc indicating that the latter contained only one-third of the protein originally present.

The method of testing sera for protective capacity has been described[1] in detail. Briefly, it is as follows: *H. pertussis* is grown on Bordet medium for 72 hours. The growth is scraped off and homogenized in 0.85% sodium-chloride solution. The turbidity of the suspension is then adjusted to match that of a standard suspension of *H. pertussis* containing 10 billion organisms per cc. Three-weeks-old Swiss mice are lightly anesthetized in a jar containing cotton saturated with a mixture of 1 part of chloroform and 2 parts of ether. From a 1 cc tuberculin syringe fitted with a short, blunt-tipped, 24-gauge needle, 0.05 cc of the standardized suspension of organisms is dropped into the nares of each mouse. Immediately after infection, 0.3 cc of the solution being tested for protective capacity is injected intraäbdominally into each mouse. This dose is repeated on each of the 2 following days. Control groups receive a comparable amount of saline.

As the mice die, they are necropsied and the degree of consolidation of the lungs is scored by a method described previously.[1] Cultures are made from the lungs and spleen. All mice surviving on the tenth day after infection are killed and examined.

The data of the first experiment, recorded in Table I, indicate the degree of protection to be expected and demonstrate that the result is statistically valid. A total of 120 infected mice were divided into 3 sub-groups of 40 each. The first group was treated in the manner already described with immune rabbit serum; the second, with normal rabbit serum; and the third, with saline. The protection afforded by the immune serum was reflected by all 3 criteria employed to measure it, *viz.*, mortality, pathological and bacteriological findings, although the bacteriological results will hardly bear statistical scrutiny. It is obvious that normal rabbit serum exerted no more protective action than did saline. It is of interest that Mishulow with

TABLE I.

Protective Value of Immune Rabbit Serum Against Experimental Murine Infection with *Hemophilus pertussis.*

Material tested	Mortality					Bacteriology				Pathology					
	No. mice	No. survived	Difference in % of survivors in the 2 groups	Standard error of difference*	Difference / Standard Error	No. mice showing positive lung cultures	Difference in % of positive lungs in the 2 groups	Standard error of difference*	Difference / Standard Error	No. of mice with "none or slight" lung lesions (0-5)	No. of mice with "moderate" lung lesions (6-14)	No. of mice with "severe" lung lesions (15-19)	Difference in % of the 2 groups showing "none or slight" lung lesions	Standard error of difference*	Difference / Standard Error
Immune rabbit serum	40	31	57.5	11.2	5.1	30	22.5	7.7	2.9	21	18	1	40	10.5	3.8
Normal rabbit serum	40	8				39				5	25	10			
Saline	40	10				32				0	24	16			

Infecting dosage of organisms = 500 million.

*Calculated by the formula $\sqrt{P_o Q_o\left(\frac{1}{N}+\frac{1}{N'}\right)}$ where P_o is average chance of death; Q_o is the average chance of survival; N is the number of mice in one group; and N′ is the number of mice in the other group. Topley and Wilson, *Principles of Bacteriology and Immunity*, second edition, Wm. Wood & Co., Philadelphia, Pa., p. 777, 1936.

TABLE II.

Comparison of Protective Power of Immune Rabbit Serum and of a Globulin Fraction Prepared from it Against Experimental Murine Infection with *Hemophilus pertussis.*

Infecting dosage of organisms	Ratio of No. of mice surviving to the No. infected			Incidence of positive lung-cultures			Incidence of No. of mice showing "none or slight" lung-lesions		
	Immune rabbit serum	Globulin fraction	Controls	Immune rabbit serum	Globulin fraction	Controls	Immune rabbit serum	Globulin fraction	Controls
1,000 million	6/6*	3/6	—	1/6	1/6	—	5/6	2/6	—
500 "	2/6	4/6*	0/6	3/6	4/6	6/6	2/6	1/6	0/6
250 "	5/6	4/6	0/6	3/6	5/6	6/6	4/6	2/6	0/6
125 "	6/6	4/6	0/6	2/6	3/6	6/6	5/6	5/6	0/6
62.5 "	5/6	5/6	0/6	2/6	1/6	6/6	5/6	5/6	0/6
31.25 "			1/6*			6/6			0/6
Total	24/30	20/30	1/30	11/30	14/30	30/30	21/30	15/30	0/30

* Endpoint of the "50% mortality dosage." In the control group this is less than 31.25 million organisms while in the immune-serum and in the globulin-fraction groups, it is calculated as 1000 million and 500 million respectively. This represents protection against at least 32 MLD for the immune serum and 16 MLD for the globulin fraction.

her co-workers,[3] using an entirely different technic, have recently reported results quite comparable to these. In her experiments, the immune serum was administered intramuscularly, while infection was produced by intraäbdominal injection of the organisms suspended in a solution of mucin.

The second experiment was designed to evaluate the protective action of a pool of immune rabbit serum (not the same one used in the first experiment) in terms of the number of MLD of the organism used, and to compare the protective capacity of the serum with that of the partially purified globulin fraction prepared from it. Accordingly, the technic of the protective test was altered by inoculating sub-groups of 6 mice each with serial 2-fold dilutions of bacterial suspension, as outlined in Table II. It was thus possible to estimate the endpoint of 50% mortality by the method of Reed and Muench.[4] The results, recorded in Table II, corroborate those of the first experiment on all points. In addition, it was estimated that the serum protected against at least 32 MLD, whereas the globulin fraction protected against 16 MLD. By all 3 of the criteria employed, there was a slight but not statistically significant difference in favor of the whole serum. One may conclude, therefore, that but little loss of protective antibody occurred during the process of fractionation. It should be noted that the titration-curve (mortality *vs.* dosage) was much less steep in the presence of antibody than it has been in control titrations where the mortality rate usually passes from 80-100% to 0-20% within a two-fold change of dosage. Such a flattening of the curve decreases the accuracy of estimation of the endpoint.

Summary. Immune rabbit serum, injected intraäbdominally into mice, protects them against experimental infection produced by the intranasal inoculation of *Hemophilus pertussis.* By precipitation in 50% saturated sodium-sulfate solution (37°C), a globulin fraction was prepared which had essentially the same protective capacity and agglutinating titer as the original serum, but only one-third of its content of protein.

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[3] Mishulow, L., Klein, I. F., Liss, M. M., and Leifer, L., *J. Immunol.*, 1939, **37**, 17.

[4] Reed, L. J., and Muench, H., *Am. J. Hyg.*, 1938, **27**, 493.

10837

The Diffusible Organic Blood-Clotting Factor.

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Larson and Greenberg[1] reported experimental evidence for the presence of a diffusible organic substance in serum which accelerates the clotting of blood. Briefly, it was found that prolonged dialysis of plasma against distilled water resulted in changes which prevented coagulation when calcium chloride was added, but the addition of a small amount of serum ultrafiltrate together with the calcium salt resulted in coagulation. This observation seemed to indicate that a diffusible substance necessary for coagulation was present in blood. The present report describes the results of experiments which were carried out in an effort to characterize the action of this factor.

Dialyzed plasma was found to vary considerably in its clotting characteristics, so purified clotting reagents were used to study the action of the filterable factor. The preparation of the reagents and the technic used in studying the activation of prothrombin have been described elsewhere.[2]

The results shown in Table I demonstrate that the ultrafiltrate factor accelerates coagulation due to a thromboplastic action, and that the active material is thermolabile.

TABLE I.
Test of Thromboplastic Activity.

0.7 ml of prothrombin solution, 0.2 ml of 0.025 M $CaCl_2$, and 0.1 ml of the solution being tested for thromboplastic activity were mixed and allowed to stand at 38°. After fixed periods of time (called "Activation Time"), the mixture was tested for thrombic activity by pipetting 0.1 ml into 1.0 ml of a fibrinogen solution or oxalated plasma diluted with 3 volumes of saline.[2] The coagulation time was noted and taken as an index of the amount of thrombin present.

A. Prothrombin + $CaCl_2$ + Saline (control):					
No activation in 10 min					
B. Prothrombin + $CaCl_2$ + Brain Thromboplastin:					
Activation Time (min)	1	2	3	4	9
Coagulation Time "	6	5.5	4.7	3.7	2.3
C. Prothrombin + $CaCl_2$ + Plasma Ultrafiltrate:					
Activation Time (min)	1.5	3	4	5.7	
Coagulation Time "	7	4	4	4	
D. Prothrombin + $CaCl_2$ + Plasma Ultrafiltrate (previously heated at 100° for one hour):					
No activation.					

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[1] Larson, C. E., and Greenberg, D. M., PROC. SOC. EXP. BIOL. AND MED., 1935, **33**, 305.

[2] Glazko, A. J., and Greenberg, D. M., *Am. J. Physiol.*, 1939, **125**, 108.

TABLE II.

Material added to 0.7 ml of dialyzed plasma and 0.2 ml of 0.025 M $CaCl_2$	Coagulation Time (min)
Saline (control)	no clot in 30 min
Saliva (heated)	5
Brain extract	4
Cephalin	6
Plasma ultrafiltrate	5

The apparent thromboplastic activity of the ultrafiltrate factor is supported by the enormous effect on the coagulation time produced by the addition of known thromboplastic materials, as shown in Table II.

Other experiments were carried out to study the effect of prolonged dialysis on the proteins involved in coagulation. The dialysis of plasma was accomplished by placing the plasma in a collodion bag together with a marble and dialyzing at 5° against running distilled water for 3 or 4 days with continuous rocking to keep the contents stirred. It was found that only a small quantity of prothrombin and fibrinogen could be isolated from the dialyzed plasma. The proteins undoubtedly were affected by the dialysis in spite of the low temperature employed. The results in Table III demonstrate the changed behavior of fibrinogen toward thrombin, due to prolonged dialysis.

TABLE III.

	Coagulation Time (sec)
Thrombin + Normal Fibrinogen	15
’’ + Dialyzed Fibrinogen	60
’’ + Dialyzed Plasma	45

Prothrombin which had been isolated from dialyzed plasma gave a very poor yield of thrombin on activation. Likewise, normal prothrombin which had been subjected to dialysis under the same conditions as the plasma was practically all destroyed, as is indicated in Table IV.

TABLE IV.

	Coagulation Time (min)
Normal Prothrombin + $CaCl_2$ + Cephalin + Fibrinogen	20
Dialyzed Prothrombin + $CaCl_2$ + ’’ + ’’	150
’’ Plasma + $CaCl_2$	120
’’ ’’ + $CaCl_2$ + Cephalin	10
’’ ’’ + $CaCl_2$ + ’’ + Normal Prothrombin	2

Summary. The diffusible substance present in blood acted as a thromboplastin in accelerating coagulation. The prolonged dialysis required to demonstrate this action produced destructive changes in the plasma factors involved in coagulation.

10838

Roentgen Ray Therapy in Pneumococcus Type I Infection in Rabbits.

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Several clinical studies suggest the value of roentgen ray therapy in the treatment of pneumococcus pneumonia. To test the effect of roentgen ray therapy on experimental pneumococcus infections under controlled conditions, the following work was done.

After the method of Goodner, 0.1 cc of a 1 :1000 dilution of an 18-hour blood broth culture of pneumococcus Type I was inoculated endermally into the shaved skin of the abdomen of each of 4 rabbits each weighing approximately 2 kg. One-half hour later roentgen ray therapy was applied to an area 6 cm x 6 cm surrounding the point of injection in 2 animals and the remaining 2 animals served as controls. Rectal temperatures were recorded daily at 9 a.m. and 5 p.m. and blood cultures were made daily from the marginal vein of the ear on the second day following injection. Seven sets of 4 rabbits were observed for 10 days following inoculation and treatment and at the end of that time if the animals were living and the temperature was normal they were considered well.

All of the factors involved in the roentgen ray radiation of these rabbits were constant, except for the time of exposure. The conditions were: 130 kilovolts, 5 milliamperes, 30 cm target skin distance, 2 mm glass filtration delivered through a 6 x 6 cm portal. With the exception of one experiment the radiation was delivered in one dose the r. units ranging from 158 to 990 r. The rabbits in this one test received 158 r. on 2 consecutive days or a total of 316 r.

Of the 14 rabbits exposed to roentgen rays 11 (78.5%) died, while only 6 (42%) of the controls succumbed. At the time of taking blood cultures 2 days after injection bacteremia was present in both treated and untreated animals. It was observed that the temperature of the treated animals usually rose to a higher point quicker than in the untreated animals, reaching a peak on the second day. Generally speaking, the lesions were characteristically different. Those exposed to roentgen rays had a more intense and widespread erythema within two days, became cyanotic and later developed wrinkling of the surrounding skin while the untreated lesions showed a somewhat more localized swelling with occasional necrotic areas. Both lesions subsided in 4 to 6 days. It was noted that almost invariably the treated animals had a more severe bacteremia, particularly in those which received the larger doses of radiation. In these animals the more severe bacteremia was paralleled by a greater mortality at an earlier date, death usually occurring 4 to 6 days after injection.

Summary. Roentgen ray therapy applied one-half hour after inoculation is ineffective in preventing bacteremia or in favorably influencing the outcome in rabbits injected intradermally with pneumococcus Type I. When larger therapeutic doses are employed roentgen rays have apparently a deleterious effect as evidenced by a more pronounced bacteremia and greater mortality.

10839 P

Vitamin B_6 Deficiency in Chicks.

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Among the members of the vitamin G complex, riboflavin[1] and pantothenic acid[2] are known to have a marked effect in promoting growth in chicks, while nicotinic acid has not been demonstrated to be needed by this species. Several recent reports[3, 4, 5] have indicated that one of more water-soluble factors, unidentified with other members of the vitamin G complex, are needed by chickens. Carter and O'Brien[6] have reported that vitamin B_6 promoted growth slightly in chicks fed a purified diet. The growth rate obtained was very low.

Previous attempts in this laboratory to investigate the vitamin B_6 requirement of the chick were hampered by the fact that a factor or factors of an unknown nature, distinct from pantothenic acid (chick anti-dermatitis factor), sharply limited the growth of chicks on purified diets. Crude concentrates of vitamin B_6 were ineffective, but growth was promoted on some diets by fat-extracted wheat germ[3] and on other diets[4] by a water extract of alfalfa meal. Subsequently it was observed that the unknown factor or factors partially escaped precipitation with phosphotungstic acid. It had been previously observed[7] that the filtrate (chick-anti-dermatitis) factor also was not precipitated by phosphotungstic acid. These observations encouraged a fresh attempt to produce vitamin B_6 deficiency in chicks.

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[1] Lepkovsky, S., and Jukes, T. H., *Science*, 1935, **82**, 326.

[2] Jukes, T. H., *J. Biol. Chem.*, 1939, **129**, 225.

[3] Lepkovsky, S., and Jukes, T. H., *Proc. Am. Soc. Biol. Chem., J. Biol. Chem.*, 1937, **119**, lx.

[4] Jukes, T. H., and Babcock, S. H., Jr., *J. Biol. Chem.*, 1938, **125**, 169.

[5] Stokstad, E. L. R., and Manning, P. D. V., *J. Biol. Chem.*, 1938, **125**, 687; Lepkovsky, S., Taylor, L. W., Jukes, T. H., and Almquist, H. J., *Hilgardia*, 1938, **11**, 559; Bauernfeind, J. C., Schumacher, A. E., Hodson, A. Z., Norris, L. C., and Heuser, G. F., PROC. SOC. EXP. BIOL. AND MED., 1938, **39**, 108.

[6] Carter, C. W., and O'Brien, J. R., *Proc. Seventh World's Poultry Congress*, 1939, p. 126.

The following basal diet was used (diet 190):

Glucose (cerelose)	63	g
Sardine meal, washed with water	20	”
Washed casein	5	”
Lard	5	”
Salt mixture	4	”
Fish oil blend, 3000 U.S.P. units of vitamin A and 400 A.O.A.C. units of vitamin D per gram	0.3	”
Yeast extract, treated with phosphotungstic acid	2	cc
Nicotinic acid*	1	mg
Riboflavin*	0.4	”
Thiamin*	0.2	”
Hexane extract of alfalfa meal, equivalent to 1 g of alfalfa meal, evaporated on the diet.		

The salt mixture consisted of bone ash, 39 parts; calcium carbonate, 20; iodized salt, 20; $MgSO_4, 7H_2O$, 8.8; KH_2PO_4, 7.5; ferric citrate, 3.5; $MnSO_4$, 1; $CuCO_3$, 0.1; $ZnCO_3$, 0.1. The yeast preparation, which had been treated twice with a large excess of phosphotungstic acid, contained 100 chick units[7] of filtrate factor (pantothenic acid) per cc. The chicks received 0.1 cc of shark liver oil, containing 14,500 U.S.P. units of vitamin A per gram, and 0.3 cc of wheat germ oil, by pipette, once weekly.

Single-comb White Leghorn chicks were kept on a regular chick-starting mash for 3 days and then placed on the experimental diets. Group 1 received basal diet 190 and Group 2 received a diet consisting of 100 g of diet 190 *plus* 0.3 mg of synthetic vitamin B_6 hydrochloride.*[8] Eight chicks were used in each group. The growth curves are shown in Fig. 1.

Nervous symptoms, consisting of various convulsive movements, were observed in 50% of the birds on the basal diet. Sometimes a bird would run about the cage in a jerky and abnormal manner. No symptoms were observed in the group receiving supplementary

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[7] Jukes, T. H., *J. Biol. Chem.*, 1937, **117**, 11.

* Generously furnished by Merck and Co., Inc., Rahway, New Jersey, through the kindness of Dr. John C. Keresztesy and Dr. Randolph T. Major.

[8] Harris, S. A., and Folkers, K., *J. Am. Chem. Soc.*, 1939, **61**, 1245.

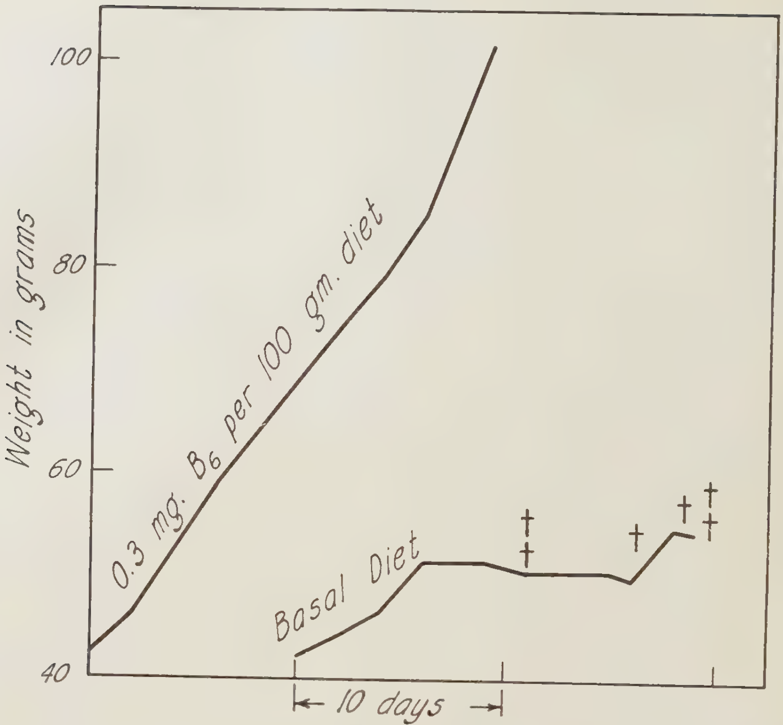

FIG. 1.

Growth curves of chicks receiving synthetic vitamin B$_6$ compared with chicks on a basal diet deficient in the vitamin. Each cross above the curve indicates the death of a chick.

vitamin B$_6$. The average daily food consumption per bird was 3.8 g for Group 1 and 6.9 g for Group 2. The efficiency of gain was 0.12 g per gram of food consumed by Group 1 and 0.35 g for Group 2. Differential blood-cell counts were made on the 17th day of the experimental period by Dr. N. M. Twisselmann of the Division of Veterinary Science, but no departures from the normal range were found in either group.

Summary. The symptoms of vitamin B$_6$ (2-methyl, 3-hydroxy, 4,5-di-(hydroxymethyl) pyridine) deficiency in chicks consist of slow growth, depressed appetite and inefficient utilization of food, followed in some cases by spasmodic convulsions and death. The symptoms were prevented by adding synthetic vitamin B$_6$ to the diet.

10840

Listerella monocytogenes in Relation to the Wassermann and Flocculation-Reactions in Normal Rabbits.

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The etiology of acute infectious mononucleosis of man is unknown. Murray, Webb and Swann[1] described a small gram-positive non-sporing bacillus, called *Bacterium* or *Listerella monocytogenes,* responsible for an acute generalized infection of rabbits characterized by large mononuclear leucocytosis with multiple focal necrosis of the liver and lymphatic glands, myocardial abscesses, and meningitis, which has been considered as a possible cause for acute infectious mononucleosis of man. Others have attempted to incriminate *Spirocheta vincenti,* while Bland[2] claims to have produced a disease in rabbits and monkeys, resembling infectious mononucleosis, with a protozoön of the genus Toxoplasma, isolated from the blood of a human case. Tidy,[3] McKinley[4] and others have thought that the disease may be due to a virus.

Whatever the cause may be, it is now well-established[5-10] that transitorily positive Wassermann reactions may occur in the disease in man and these may be independent of the production of antibodies (agglutinins and hemolysins) for sheep erythrocytes.

Under the circumstances we thought it advisable to determine whether or not *Listerella monocytogenes* was capable of producing in rabbits the reagin or antibody-like substance responsible for the Wassermann and various flocculative reactions occurring in syphilis. If such occurred it would lend some support to the assumption that this organism may be in etiological relationship to acute infectious mononucleosis of man and at all events prove useful for determining whether or not the bacillus carried an antigen capable of producing non-specific Wassermann and flocculative reactions.

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[1] Murray, E. G. D., Webb, R. A., and Swann, M. B. R., *J. Path. and Bact.*, 1926, **29**, 407.

[2] Bland, J. O. W., *Brit. J. Exp. Path.*, 1931, **12**, 311.

[3] Tidy, H. L., *Lancet,* 1934, **2**, 236.

[4] McKinley, C. A., *J. A. M. A.*, 1935, **105**, 761.

[5] Löhe, H., and Rosenfeld, H., *Dermat. Z.*, 1928, **53**, 373.

[6] Radford, M., and Rolleston, J. D., *Lancet,* 1930, **2**, 18.

[7] Weber, F. P., and Bode, O. B., *Münch. Med. Wchn.*, 1931, **78**, 1598.

[8] Gooding, S. E. F., *Practitioner,* 1931, **127**, 468.

[9] Bernstein, A., *Am. J. Med. Sci.*, 1938, **196**, 79.

[10] Hatz, B., *Am. J. Clin. Path.*, 1938, **8**, 39.

Two cultures were employed kindly furnished by the American Type Culture Collection, namely, No. 4428, isolated by Murray[1] from the spontaneous mononucleosis of rabbits, and No. F.302 (source not stated).

Eight rabbits were selected giving negative preliminary Wassermann reactions according to the method of Kolmer;[11] one of these gave a weakly positive Kahn (standard) and 2 weakly positive Kline (diagnostic) reactions.

Four of the rabbits were then given 6 intravenous injections of approximately 40 million living bacilli of strain 4428 per kilo every 5 to 7 days and 4 additional animals were given similar doses of heat-killed organisms (60°C for 1 hour).

One week after the last inoculation the serological tests were repeated. All 8 rabbits gave negative Kolmer complement-fixation reactions. The 7 giving preliminary negative Kahn reactions gave negative reactions after immunization as likewise the 6 which gave negative Kline reactions before immunization.

Eight additional rabbits were selected giving preliminary negative Kolmer reactions; 4 of these gave positive Kahn (standard) and Kline (diagnostic) reactions.

Four of the rabbits were then given 6 intravenous injections of approximately 40 million living bacilli of strain F.302 per kilo every 5 to 7 days and 4 additional animals were given similar doses of heat-killed organisms (60°C for 1 hour).

One week after the last inoculation the serological tests were repeated. All 8 rabbits gave negative Kolmer complement-fixation reactions. The 4 giving preliminary negative Kahn reactions gave negative reactions after immunization as likewise the 4 which gave negative Kline reactions before immunization.

It would appear, therefore, that the immunization of normal rabbits with the two strains of *Listerella monocytogenes* (4428 and F.302) employed did not produce the reagin responsible for complement-fixation and flocculative reactions with antigens commonly employed for the serum-diagnosis of syphilis. Whether or not the negative results were due to the absence of an antigen or other substance capable of producing the antibody or reagin involved in the complement-fixation and flocculative reactions observed in syphilis, we are unable to state. At least the immunization of normal rabbits with living and heat-killed organisms of the two particular strains employed in this study did not produce detectable amounts of the reagin or antibody concerned in the serum-tests for syphilis.

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[11] Kolmer, J. A., and Boerner, F., *Approved Laboratory Technic*, D. Appleton and Company, second edition, 644, 1938.

Preliminary macroscopic agglutination-tests were conducted with the bacilli of both strains before immunization in final dilutions of serum ranging from 1:10 to 1:320 and an incubation of 2 hours at 56°C followed by refrigeration over night, but with completely negative results. The tests were repeated one week after the sixth inoculation and the results showed that all produced agglutinins for homologous strains in final dilutions of serum ranging from 1:80 to 1:1280. As a general rule the titers were somewhat higher with both strains with the sera of rabbits immunized with living bacilli.

Preliminary complement-fixation tests were conducted with the sera of all rabbits employing as antigens heat-killed suspensions of both strains in saline solution in dose corresponding to one-fourth their anticomplementary units, according to the method of Kolmer;[11] all gave completely negative reactions. One week after the sixth inoculation the tests were repeated and all gave positive reactions of varying degree with their homologous antigens, indicating that both strains were antigenic in the production of complement-fixing antibody but, as previously stated, without producing complement-fixing antibody for antigens of alcoholic extracts of beef heart commonly employed in the serum-tests for syphilis.

The sera of all rabbits were tested for agglutinin for sheep corpuscles before and after immunization according to the technic of Davidsohn with human sera,[12] but all gave negative reactions in final dilutions of serum ranging from 1:7 to 1:7168, indicating that both strains apparently did not carry the heterophilic antigen producing antisheep agglutinin.

All of the rabbits withstood immunization with but little loss of weight. At necropsy none showed focal necrosis of the liver, enlargement of lymphatic glands, or evidences of meningitis, indicating that both strains were avirulent for these animals.

Three preliminary total and differential leucocyte counts were made on all animals before immunization and thereafter 5 days after each inoculation. All showed slight to moderate leucocytosis after 6 inoculations mainly due to an increase of polymorphonuclears. The monocytes were slightly increased in some of the animals.

Summary. (1) The intravenous immunization of rabbits with living and heat-killed avirulent strains (4428 and F.302) of *Lis-*

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[12] Ref. 11, page 110.

terella monocytogenes did not produce complement-fixing or flocculating reagins for antigens commonly employed in the serum-diagnosis of syphilis. (2) Both produced complement-fixing antibody and agglutinins for antigens of homologous strains of *Listerella.* (3) Neither strain produced agglutinin for sheep erythrocytes in the immunization of rabbits. (4) Both strains produced moderate leucocytosis mainly due to an increase of polymorphonuclears with a slight increase of the monocytes in some of the animals.

10841

General Anesthesia by Cooling.

G. H. PARKER.

From the Biological Laboratories, Harvard University.

It is often desirable to induce in animals a general anesthesia for operative purposes without the use of drugs. This is particularly true where, as in the study of chromatophores, the effectors are especially open to drug stimulation and may remain responsive to minute amounts of the reagent for relatively long periods of time. As an effective means to a drugless anesthesia cold is very convenient. Fishes, amphibians, and reptiles may be immersed in cracked ice and water or simply in cracked ice till they are thoroughly chilled, which occurs ordinarily in from 10 to 15 minutes. They will then remain quiescent either on an operating board or on a bed of cracked ice till an operation can be performed. From this treatment the animals recover quickly at room temperatures and are soon quite free from any after-effects of the cold. They may, therefore, be tested, if this step is desirable, almost at once, for they contain no residual reagent, the complete disappearance of which must be awaited before further work can be done.

For some time fresh-water and salt-water fishes have been treated successfully in the Harvard Laboratories by this method (Parker,[1] Abramowitz,[2] Osborn[3]). Recently Abramowitz has employed it on a larger scale in that dogfishes as much as a meter in length have been immersed in a tub containing cracked ice and seawater and there stupefied preparatory to an operation. Frogs are of course easily anesthetized either in cracked ice or in ice and water (Parker and Scatterty[4]), and lizards may be chilled with cracked ice (Parker[5]). Thus far we have treated only cold-blooded vertebrates, but Pfeiffer[6] has applied the method to newly-born rats. For some time after birth the young rat fails to maintain a high, constant temperature. During this period Pfeiffer found that it could be chilled in an electric refrigerator and after it had become motionless it could be satisfactorily operated upon on a bed of ice. On warming such a rat, it again became active and was received by its mother. This procedure appears to have been first used by Wiesner.[7]

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[1] Parker, G. H., *Proc. Am. Philos. Soc.*, 1937, **77**, 223.

[2] Abramowitz, A. A., *Science*, 1937, **85**, 609.

[3] Osborn, C. M., *J. Exp. Zool.*, 1938, **79**, 309.

10842 P

Relation of Concentrations of Free to Conjugated Sulfapyridine in the Blood of Patients.

ARMINE T. WILSON, DELORES KAELY AND GLENN E. CULLEN.

From the Children's Hospital Research Foundation and the Department of Pediatrics, University of Cincinnati, Ohio.

In the course of a clinical investigation of the use of sulfapyridine in the treatment of pneumonia[1] and other conditions, we were considerably interested in the practical problem of dosage in relation to the level of the drug obtained in the blood. Patients were given varying amounts of the drug according to weight, 1 or 1.5 grams per lb per 24 hr, in order to study their blood levels and to compare the influence of various levels on therapeutic response. Six hundred and fourteen determinations* were made of free sulfapyridine content of the blood of 126 children. On the basis of the findings of these determinations we concluded, as will be reported elsewhere, that *groups* of patients of various ages, receiving equivalent amounts of sulfapyridine according to their weights have, on the average, approximately the same levels of free drug in the blood; but that the individual patients constituting these groups vary widely in their levels so that it becomes necessary to adjust the dosage of any given case to reach the level desired.

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[4] Parker, G. H., and Scatterty, L. E., *J. Cell. Comp. Physiol.*, 1937, **9**, 297.

[5] Parker, G. H., *J. Exp. Biol.*, 1938, **15**, 48.

[6] Pfeiffer, C. A., *Am. J. Anat.*, 1936, **58**, 195.

[7] Wiesner, B. P., *J. Obst. Gynœcol. Brit. Empire*, 1934, **41**, 867.

* Marshall's method adapted for use with the Evelyn colorimeter was used in making the determinations.

Merck and Co. kindly furnished the sulfapyridine (Dagenan).

[1] Wilson, A. T., Spreen, A. H., Cooper, M. L., Stevenson, F. E., Cullen, G. E., and Mitchell, A. G., *J. A. M. A.*, 1939, **112**, 1435.

Many factors were considered in possible explanation of this individual variability. It seemed probable that differences in absorption of the drug, in fluid turn-over, in the acid-base reaction of the gastro-enteric tract, in the power to conjugate the drug, and in the occurrence of vomiting and diarrhea all contributed their share in producing this variation.

It has been suggested[2] that patients vary in their ability to conjugate sulfapyridine, and that this variation might be a major factor in accounting for differences in level of the free drug from patient to patient. If this were true, then it would follow that the total drug levels should be constant, relatively speaking, for the various patients, and that those who conjugated a large part of the drug would have levels of the free drug lower to that extent, while those who conjugated little of the drug would have correspondingly higher free drug levels. That this is not correct is shown by the findings in 100 determinations of free and total sulfapyridine levels on the blood of 26 of our patients. There was indeed greater variation in the total drug levels of these patients (3.5 to 28.5 mg per 100 cc of blood) than there was in their free drug levels (1.4 to 17.4).

We observed a marked tendency for the level of acetylsulfpyridine in the blood of patients receiving varying amounts of sulfapyridine to be around 2 to 3 mg per 100 cc regardless of the level of the total or of the free drug in their blood. This was seen to be true of almost all patients except for a few of those having unusually high total drug levels. Three of these showed occasional samples with levels of 6 to 8 mg per 100 cc and, in the one case, of 11 mg per 100 cc. This case with the highest level shown on the chart had only one sample analyzed. The accompanying diagram, in which the patients have been arranged in order of the average of their total sulfapyridine blood levels, illustrates this point. We have considered the possible rôle of the relative insolubility of acetylsulfpyridine in the blood, the possibility of a variable renal clearance, and the possibility of a definitely limited ability to produce the conjugated drug in explanation of this phenomenon, but the explanation is not immediately apparent. It is possible that the sudden rise in conjugated drug content of the blood with increasing dosage may serve as a warning of danger, particularly since the acetylated compound has been

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[2] Meakins, J. C., and Hanson, F. R., *Canad. M. A. J.*, 1939, **40**, 333.

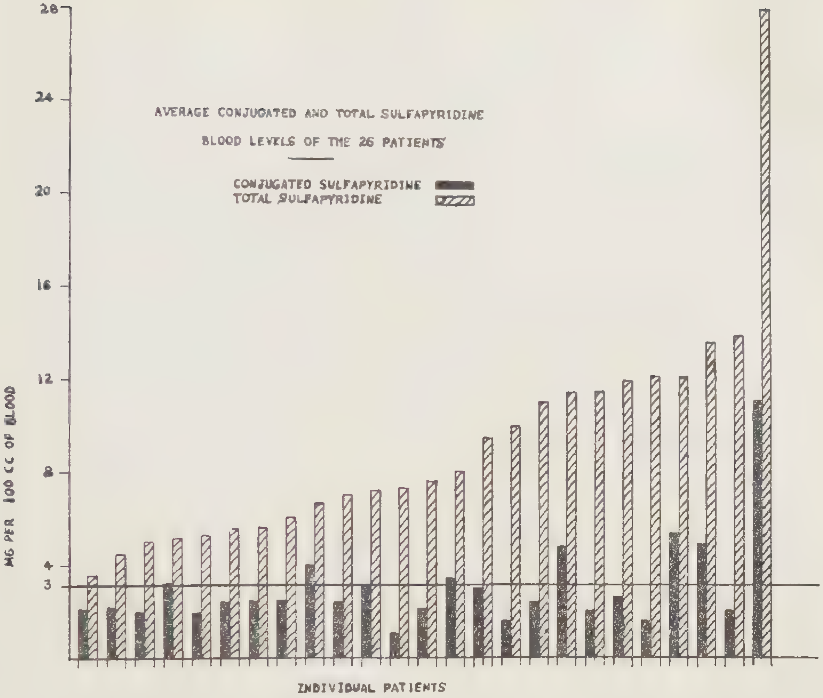

FIG. 1.

This diagram shows that the average blood levels of conjugated sulfapyridine tend to remain below 3 mg per 100 cc, independently of the amount of free and total drug present, except in a few cases with high total levels.

shown to be potentially harmful both from direct toxic action[3] and from its precipitation in crystalline form in the ureters.[4]

Conclusion. Patients show wide variation in blood content of free and total sulfapyridine when receiving standard dosage by weight, but the conjugated drug in most cases is present in about 2 to 3 mg concentration per 100 cc blood. However, in a few instances, patients with high levels of free sulfapyridine depart from this tendency, and have high conjugated drug content in the blood.

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[3] Marshall, E. K., Bratton, A. C., and Litchfield, J. T., *Science*, 1938, **88**, 599.

[4] Antopol, W., and Robinson, H., PROC. SOC. EXP. BIOL. AND MED., 1939, **40**, 428; Gross, P., Cooper, F. B., and Lewis, M. L., *ibid.*, 1939, **40**, 448.

10843

A Mechanical Respirator as an Adjunct to Closed System Anesthesia.*

FREDERICK R. MAUTZ. (Introduced by C. S. Beck.)

From the Laboratory of Surgical Research, Western Reserve University School of Medicine, and the University Hospitals, Cleveland.

The average operating room makes no provision for mechanically maintaining artificial respiration in patients during operation. In the absence of adequate pulmonary ventilation, other resuscitative measures are generally of little value in advanced respiratory or cardiac failure.[1] Such accidents are sufficiently common in the operating room to warrant a carefully organized plan for resuscitation and especially to include in this plan a method of artificial respiration for the surgical patient.

During the past 2 years a mechanical respirator, of a type commonly used in physiological laboratories for the intermittent insufflation of compressed air down a tracheal catheter, has been kept available on the operating floors of the University Hospitals. During this period of time the respirator has been successfully used in 4 cases of acute cardio-respiratory failure and might have been successful in several other cases if the artificial respiration could have been instituted more promptly. This fact aroused the interest of the author in developing a simple, dependable mechanical respirator as an integral part of the commonly used anesthesia machines.

Since the introduction of carbon dioxide absorbing technic and the use of a tight fitting face mask, creating a closed lung-bag system, the simplest effective method of artificial pulmonary ventilation in the event of inadequate spontaneous respirations is by rhythmic manual compression of the rebreathing bag. Utilizing this principle, the following apparatus was constructed.† (Fig. 1.)

The rebreathing bag of the anesthesia machine is enclosed in a rigid airtight container, B, made of pyralin, a nonbreakable plastic, and it is attached to the soda-lime canister A, in this case, of a Heidbrink anesthesia machine. Air under pressure, from any source 8, is intermittently injected into the space between the rigid container

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* Aided by a grant from the Alumnæ Association of the University Hospitals School of Anesthesia, Cleveland, Ohio.

[1] Mautz, Frederick R., PROC. SOC. EXP. BIOL. AND MED., 1937, **36**, 634.

† This apparatus was built by Mr. Morris Dann of the Department of Pharmacology of the Western Reserve University School of Medicine. The injector unit C was designed and has been built since 1928 by Mr. Dann.

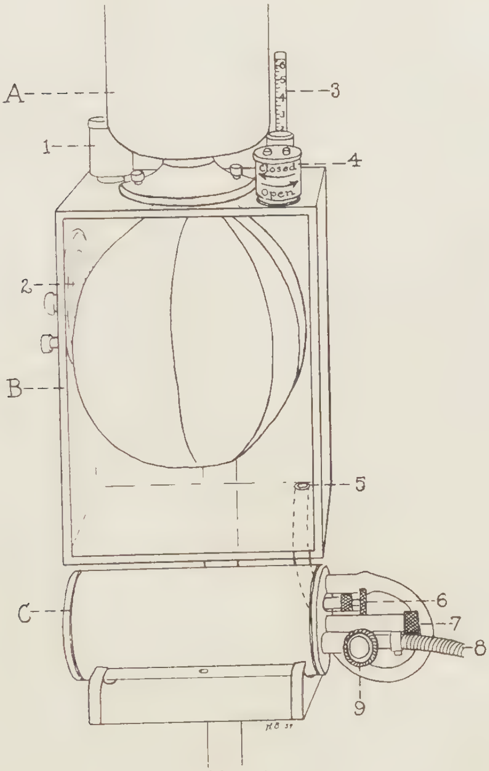

Fig. 1.

and the rebreathing bag through inlet 5 which can be controlled as to rate and volume by valves 9 and 6. Between injections the space around the bag is automatically opened to the atmosphere through the exhaust valve 7. A mercury manometer, 3, measures the pressure developed around the rebreathing bag. A safety pop-off valve, 1, prevents the development of excessive pressures. A removable window, 2, gives easy access to the rebreathing bag. A hand controlled exhaust valve, 4, makes it possible to open the space surrounding the bag to the atmosphere and thus completely eliminate the compressing effect of the injector, even though it be in operation. This valve acts to engage or disengage the respirator mechanism at will and if just slightly opened it gives a delicate control of peak pressures developed during the air injection. This control makes it possible to bring the respirator into use in a fraction of a second without any change in equipment. The pneumatic control of bag compression is extremely flexible and easy to regulate.

This respirator attachment has been used clinically on a number of patients both with chest intact and also in the presence of an open pneumothorax. With chest intact the intermittent pulmonary inflations are accepted without a struggle providing the patient's respirations are only moderately depressed and even though the inflations are not synchronous with spontaneous respirations. The air volume of the rebreathing bag and pyralin container acts as a buffer for any transfer of force from a source of compressed air to the lung. In the presence of a free airway and a tight fitting face mask no tracheal catheter is necessary; however, it may be desirable in many situations to have an intratracheal catheter in place, since if there is obstruction air might be injected into the stomach. However, with a free airway and pressures not in excess of 15 mm Hg this did not occur in our cases.

This apparatus would seem of particular value in open pneumothorax. We have had an opportunity to use the respirator in several patients with open pneumothorax and found a very satisfactory, readily controlled expansion of the lung, with the maximal pressure not exceeding 10 mm Hg, less pressure being required in the presence of open pneumothorax than with the chest intact to produce a given exchange.

With this arrangement the surgeon can produce a bilateral open pneumothorax and yet maintain any desired degree of pulmonary ventilation. In open pneumothorax there was no difficulty due to failure of the lung to collapse between inflations. This is a strong argument against the need of suction, as is used in some commercial resuscitators. In the presence of fluid in the air passages this should be removed by means of catheter suction. Under such circumstances there might be a distinct hazard to positive pressure insufflation in spreading infection to normal lung, but of course in respiratory failure even this would not contraindicate the use of such a respirator.

Simultaneously with this study Crafoord[2] in Sweden was using intermittent pulmonary insufflation in a large series of pneumonectomies. The mechanical arrangement for accomplishing this was different but in principle the arrangement is essentially that incorporated in this simplified apparatus. There are opportunities for further mechanical development of this principle so as to make such a respirator available in operating rooms that do not have compressed air lines. The present apparatus would run several hours on a large cylinder of oxygen or compressed air.

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[2] Crafoord, C., *Acta chir. Scandinav.* (supp. 54), 1938, **81**, 1.

10844

Action of Apomorphine Hydrochloride on the Intact Intestine in Unanesthetized Dogs.*

CHARLES M. GRUBER, VICTOR G. HAURY AND MILES E. DRAKE.
From the Department of Pharmacology, Jefferson Medical College, Philadelphia, Penn.

Gruber and Brundage[1] published the results of 18 experiments performed on 8 dogs with Thiry-Vella loops of the small intestine using apomorphine hydrochloride. In these the drug produced an increase in general tonus in all of the observations made. In some, of which Fig. 1 in their article is an example, the sudden increase in the general tonus was followed by a rather prolonged decrease in the general tonus of the gut. This temporary increase, followed by a prolonged loss in general tonus was noted in the 8 experiments performed on the 3 animals in which the Thiry-Vella loops were of the jejunum, but was not observed in those experiments in which the ileum was used. Since their findings were reported, Slaughter and Gross[2] have presented the results of 10 similar experiments performed on 3 dogs with Thiry-Vella loops of the ileum. In these they observed only a decrease in the general tonus of the gut, whether the drug was injected intravenously or subcutaneously. In some of their experiments complete cessation of the contractions was noted. Because of the discrepancy between the results of the above investigators, additional observations seemed in order.

Six female dogs, weighing from 8.2 to 18.6 kg were used in this research. Except for the fact that in 5 of 6 animals, after the segment of intestine had been detached, the continuity of the alimentary canal was reëstablished by end to end anastomosis, the operation and experimental procedures were the same as those previously described by one of us.[3] In all cases the animals had been without food for 24 hours previous to the experiment, and a week was allowed to elapse between each 2 experiments on the same animal. The drug was administered intravenously to unanesthetized animals.

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* This research was made possible by a grant from Parke, Davis and Company to the Department of Pharmacology in Jefferson Medical College for research in science.

[1] Gruber and Brundage, PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 863.

[2] Slaughter and Gross, *J. Pharm. and Exp. Therap.*, 1938, **63**, 289.

[3] Gruber and Brundage, *J. Pharm. and Exp. Therap.*, 1935, **53**, 122.

The apomorphine hydrochloride employed in these experiments was the same as that used by one of us[1] in a previous report and complied with all of the purity tests given in the United States Pharmacopoea XI. In addition to this, through the generosity of Dr. O. H. Plant,‡ we were able to make observations with a sample of the same apomorphine as that employed by Slaughter and Gross[2] in their investigations.

Immediately before each injection, the drug was weighed and dissolved in distilled water so that each cubic centimeter contained 1 mg of apomorphine hydrochloride. The dose administered in each case was 0.02 mg per kg body weight.

Results. Twenty-two experiments were performed on the 6 animals used. In 10 experiments an unmistakable increase in the general tonus occurred and in some of these this increase lasted over 38 minutes. In 5 a decrease only in the general tonus was observed, and in the remaining 7 experiments the results were regarded as doubtful. In these doubtful cases usually an increase in general tonus occurred, which was followed shortly by a decrease which again changed to an increase in the general tonus, *e. g.,* in one experiment the increase in general tonus was of 5 minutes' duration, the subsequent decrease of 8, and the following increase of 21 minutes. In another instance the increase in the general tonus following the injection lasted 13 minutes and the subsequent decrease was 41 minutes long. From this investigation there seems to be a difference in the responses of dogs to apomorphine. Moreover, for no apparent cause, the same dog may respond differently to the drug on different days.†

In one of our animals 3 injections of the apomorphine were made and a decrease in the general tonus of the gut was noted in each instance, even though borborygmi were strongly audible. After 4 injections in each of 2 other animals, only once in each animal was an unmistakable increase in the general tonus of the gut recorded. These increases were obtained after the injection of the sample obtained from Dr. O. H. Plant. With the use of this sample of apomorphine, 4 of the 6 dogs showed increased tonus of the gut following its administration and one of the remaining was doubtful. (See Fig. 1.)

One dog responded invariably to apomorphine by contraction,

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‡ The authors wish to thank Dr. O. H. Plant for his cooperation in supplying some of the apomorphine used in this investigation.

† Necropsies of these animals showed that (1) in 2 the loops were excised 10 cm and 35 cm above the ileocecal valve; (2) in 2 others, 91 and 96 cm above the ileocecal valve and 152 and 144 cm respectively from the stomach, and (3) in the remaining 2 animals the loops were removed 125 and 152 cm from the ileocecal valve and only 116 and 91 cm respectively from the stomach.

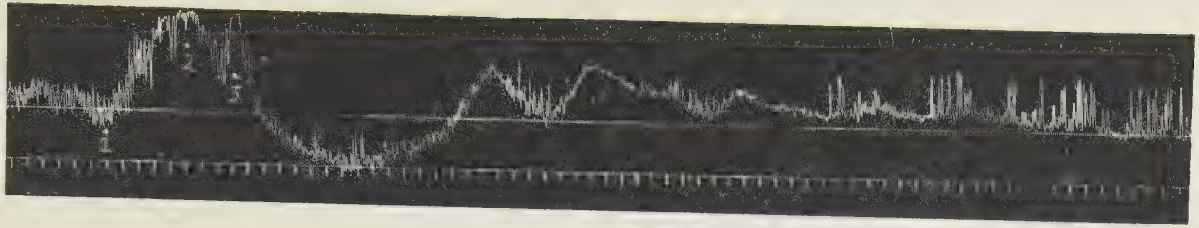

FIG. 1.

Unanesthetized dog. Weight 8.2 kg. Top curve is that of the contractions of the small intestine using a rubber balloon in the Thiry-Vella loop and 15 cm water pressure within the balloon. Bottom record the time in intervals of 30 sec. 1, Apomorphine hydrochloride (0.16 mg) injected into the saphena para vein. 2, Animal nauseated. 3. Animal vomited.

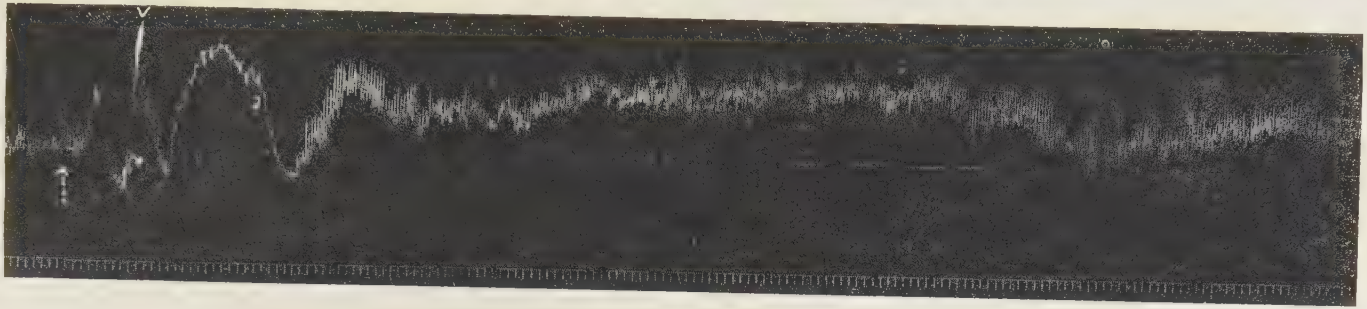

FIG. 2.

Unanesthetized dog. Weight 18.2 kg. Top curve, contractions of the small intestine (See Fig. 1) and the bottom record the time in intervals of 10 seconds. 1, Apomorphine hydrochloride (0.36 mg) injected intravenously. V, Animal vomited. 2, Animal again showed signs of nausea but neither retched nor vomited. A normal control record, showing no change in general tonus, of 45 min duration had been taken before the drug was injected.

and the remaining 2 usually responded by contraction, though in 3 experiments the results again were doubtful.

Fig. 1 illustrates what we regard as one of the doubtful findings. In this experiment the animal weighed 8.2 kg and received, at 1, 0.16 mg apomorphine hydrochloride intravenously. There is seen a sudden increase in the general tonus of the gut which requires approximately 4 minutes to return to the control level. At 2, in the figure, the animal first showed the central effects of apomorphine; nausea, salivation, swallowing and licking reflexes. At 3, the animal vomited. The sudden increase in the general tonus here is mechanical, due to the contractions of the abdominal muscles during retching. Following this, the animal continued to be nauseated. It is during this period that the general tonus of the gut falls below the control level. After the general tonus remains below the control level for 7 minutes it rises again and remains above normal level for about 20 minutes.

Fig. 2 is presented as typical of all the experiments in which an increase in the general tonus was produced by apomorphine. The animal used in this experiment weighed 18.2 kg. After a control record of 45 minutes' duration showed no fluctuation in general tonus of the gut, 0.36 mg of apomorphine hydrochloride were injected intravenously at 1. A sudden increase in the general tonus occurs during the next 20 seconds, which is followed by an equally rapid fall during the period of induced nausea, as shown by licking reflex, salivation, etc. At V the animal retched and vomited and the sudden increase in the general tonus is due to the contractions of the abdominal muscles. At 2, the animal again salivated excessively, and showed the licking and swallowing reflexes but did not vomit. In this, as in all of our experiments, the animal subsequently became depressed, drowsy and slept. In this animal the general tonus of the gut remained above the control level for over 16 minutes.

From the results of these experiments and those previously recorded it is apparent that apomorphine can cause an increase in the general tonus of the small intestine in some unanesthetized dogs. The action, though similar to that of morphine, is not as abrupt, pronounced nor as prolonged as that caused by morphine. These experiments also show that the small intestine of some unanesthetized animals may invariably respond to apomorphine by a loss in general tonus. However, even in some of these cases, the presence of borborygmi and the augmented peristaltic contractions of the segment would indicate that the activity of the gut had been increased.

Summary and Conclusions. 1. The results of these experiments support the findings published by Gruber and Brundage[1] and by Slaughter and Gross.[2] 2. Apomorphine when injected intravenously may either increase or decrease the general tonus of the intact intestine, depending upon the animal and the condition of the animal at the time of the injection. Some animals respond to apomorphine only by increased tonus, others by decreased tonus, and still others by either or both responses. 3. The peristaltic contractions may be augmented by apomorphine especially when the general tonus is diminished. 4. Borborygmi are commonly noted following the intravenous injection of apomorphine.

10845

Hydrolysis of Acetycholine by Turtle Blood.*

J. M. Little. (Introduced by W. E. Garrey.)
From the Department of Physiology, Vanderbilt University.

In connection with studies being conducted in this laboratory on the physiology of the turtle heart, it was of interest to know the choline-esterase activity of turtle blood. Work already done on the blood of other forms has revealed marked differences in the hydrolysis of acetylcholine between whole blood and serum and further variations depending upon the species. Galehr and Plattner[1, 2] found that human whole blood, shadow cells and washed corpuscles have a greater hydrolytic action on acetylcholine than the serum has. The filtrate from an acetylcholine solution treated with animal charcoal was from 78-100% physiologically inactive, but the total activity could usually be restored by acetylation. By analogy to the charcoal effects they concluded that the hydrolysis of acetylcholine by blood was due to surface catalysis. On the other hand, Engelhart and Loewi[3] repeated the charcoal experiments of Galehr and Plattner and found that partial inhibition of the destruction of acetylcholine could be obtained with a 1:5,000 dilution of physostigmine, which had been shown to be effective in preventing acetylcholine hydrolysis by extracts of frog hearts, and complete inhibition

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* Supported in part by a grant from a Fluid Research Fund furnished by the Rockefeller Foundation.

[1] Galehr, O., and Plattner, F., *Pfluger's Arch.*, 1927, **218**, 488.

[2] *Ibid.*, 1927, **218**, 506.

[3] Engelhart, E., and Loewi, O., *Arch. exp. Path. u. Pharm.*, 1930, **150**, 1.

with equimolar concentrations of strychnine. They showed that physostigmine also completely prevented this hydrolysis by defibrinated beef blood in much greater dilutions (1:30,000) and by human serum in dilutions as great as 1:400,000. Strychnine did not have this effect on blood. When beef blood was heated at 58°C its hydrolytic activity was lost while human blood retained its activity when treated in the same manner. However, it was shown that although dried human blood retained its hydrolytic activity, it was lost after heating at 58°C. On the basis of these results, they concluded that the acetylcholine-splitting activity of human and beef sera, plasma, erythrocytes and stromata is due to the presence of an enzyme rather than to surface catalysis, and that this enzyme is not necessarily different in beef and human blood since drying of the blood might inactivate a protective agent. Ammon and Voss[4] found that human, rabbit and beef sera had less activity than whole and laked blood, while dog and horse sera had more activity than whole and laked blood. There was complete inhibition with physostigmine in all these preparations. Their findings on dog and horse sera do not conform to the view of Galehr and Plattner. Stedman and Stedman[5] investigated defibrinated blood, laked and unlaked, sera and corpuscles in a variety of species. They found that human, horse, guinea pig, pig and rabbit sera and corpuscles showed activity; in the cat, fowl and duck only the sera showed activity; while in the ox, sheep and goat, corpuscles alone showed activity. There was no difference in laked and unlaked blood. Physostigmine inhibition was not tried.

Method. In this work *Pseudemys elegans* was used. Blood was obtained by decapitation and was oxalated or allowed to clot as desired. Determinations of the hydrolysis of acetylcholine were made of 0.5 cc samples, using a modification of the Glick[6] method. The sample was added to 5 cc of water containing 20 mg of acetylcholine bromide with or without 0.10 mg of Prostigmin, the volume being made to 10 cc with distilled water (Prostigmin dilution of 1:100,000). When unlaked blood was examined, Ringer's solution replaced the water. The solution was adjusted to pH 8.0 and maintained there for 20 minutes by the addition of 0.01N NaOH from a microburette, using a glass electrode and gently stirring with a stream of air bubbles. All determinations were made at room temperature.

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[4] Ammon, R., and Voss, G., *Pfluger's Arch.*, 1934, **235**, 393.

[5] Stedman, Edgar, and Stedman, Ellen, *Biochem. J.*, 1935, **29**, 2107.

[6] Glick, D., *Biochem. J.*, 1937, **31**, 521.

Results. The results are expressed in Table I in cc of 0.01N NaOH neutralized by the acetic acid liberated upon hydrolysis of acetylcholine by 1.0 cc of sample during 20 minutes. Detectable spontaneous hydrolysis of the ester did not occur in distilled water, while in Ringer's solution this amounted to 0.12 cc of 0.01N NaOH; the tabulated results have been corrected for this. It will be seen that laked whole blood has considerable choline-esterolytic activity, while serum and plasma show none in some cases and only traces in others, which may be due to slight hemolysis. Prostigmin completely inhibits the activity of laked blood and plasma. Unlaked whole blood has from 18-59% more activity than laked whole blood and this difference is not inhibited by Prostigmin. Since there is no difference in activity between unoxalated serum and oxalated plasma, the oxalate had no effect on the choline-esterolytic activity. Several experiments with unlaked whole blood alone showed that there was no glycolysis under these conditions and that the acid developed was due solely to the hydrolysis of acetylcholine.

The hydrolytic activity of turtle blood towards acetylcholine was found to be quantitatively as variable as that of other forms and apparently this activity is related solely to the corpuscular elements. It is of interest to note that of the 3 forms investigated which have nucleated erythrocytes the fowl and duck have activity only in serum, while the turtle blood activity is due to the corpuscles. A part of the hydrolysis may be ascribed definitely to the presence of choline-esterase since Prostigmin has some inhibitory effect, but there remains a

TABLE I.
Results Expressed in cc of 0.01N NaOH Neutralized by the Hydrolysis of Acetylcholine.

		Whole Blood				
			Unlaked			
No.	Plasma, Normal	Laked, Normal	Normal	With Prostigmin	Hæmatocrit, % cells	Cells
1	.30*					
2	.34*	1.27				
3	.06*	0.31				
4	.00	0.33			25.3	1.31
5	.00	1.84†			23.1	7.97
6	.10†	1.99†			34.1	5.82
7	.07	1.06†			23.5	4.59
8	.22†	0.81†	0.96	0.12	14.5	5.54
9	.06	1.76†	2.16	0.45	22.2	7.93
10		2.57	3.72	1.18		
11		1.60	2.54	0.92		

* Serum.

† Showed no hydrolysis with added prostigmin.

considerable portion of the activity which is not due to esterase since it is not inhibited by Prostigmin. Furthermore, this fraction of the activity is dependent upon the integrity of the corpuscles since it is not present after laking the blood. The greater activity of unlaked blood is not due to the presence of Ringer's solution since it is not a constant value as would be expected if this were true. These results indicate that in turtle blood some of the hydrolytic activity towards acetylcholine may be due to corpuscular surface catalysis. If one is to assume enzymatic hydrolysis of acetylcholine it would appear essential to demonstrate, as a control, that it is completely inhibited by physostigmine or Prostigmin.

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Further Evidence for a Mammogenic Factor in the Rat Hypophysis.

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Gomez, Turner, and Reece[1] reported that the mammary glands of hypophysectomized male guinea pigs could be developed by the implantation of the pituitaries of estrogen-treated male rats but that no mammary growth resulted from implantation of normal male rat pituitaries. Gomez and Turner[2] reported a confirmation and extension of these results. The existence of the "mammogenic factor" described by the aforementioned authors seems to have been demonstrated in pregnant cattle pituitaries when tested on castrated rabbits[3] and rats[3] and normal mice.[4, 5] Nelson[6] reported recently that the mammary development of hypophysectomized immature female rats, implanted with pituitaries from estrogen-injected rats of either sex, did not exceed or even equal that which obtains with the implantation of normal pituitaries. He also states that he failed to confirm the claim for a specific "mammogenic hormone". Since the existence of a hypophyseal "mammogenic factor" seems in question, an attempt was made to determine whether such a factor was present in rat hypophyses, when tested on hypophysectomized castrated rats. The results are reported herewith.

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* Aided by a grant from American Philosophical Society, Penrose Fund. Dr. E. Schwenk, Schering Corporation, generously supplied us with Progynon-B. Also we wish to thank Dr. W. C. Russell for the donor rats.

† Journal Series paper of the New Jersey Agricultural Experiment Station, Department of Dairy Husbandry.

[1] Gomez, E. T., Turner, C. W., and Reece, R. P., Proc. Soc. Exp. Biol. and Med., 1937, **36**, 286.

[2] Gomez, E. T., and Turner, C. W., *Mo. Agr. Exp. Sta. Res. Bul.*, 1937, 259.

[3] Gomez, E. T., and Turner, C. W., Proc. Soc. Exp. Biol. and Med., 1938, **37**, 607.

[4] Lewis, A. A., and Turner, C. W., Proc. Soc. Exp. Biol. and Med., 1938, **39**, 435.

[5] Lewis, A. A., Turner, C. W., and Gomez, E. T., *Endocrinology*, 1939, **24**, 157.

[6] Nelson, W. O., *Anat. Rec.*, 1938, **72**, 117 (Suppl.).

Sexually immature female rats of uniform age and weight were castrated and hypophysectomized. Five days later, these rats received 11 daily implants of one hypophysis from normal and estrogen-treated rats of both sexes as follows: 3 received implants from adult females, 2 received implants from adult males, 5 received implants from estrogen-treated females (20 R.U. Progynon-B for 10 days), and 1 received implants from similarly estrogen-treated males. Five castrated hypophysectomized immature females were the controls. During the treatment daily body weighings were made and at autopsy 12 days later, the uteri, adrenals, and thyroids were removed and weighed. Mammary glands were removed and studied as whole mounts. No pituitary fragments were found in the hypophysectomized rats.

The mammary glands of the 5 animals that received no treatment following hypophysectomy were involuted, and consisted mainly of a naked duct system. The mammary glands of all the 11 animals receiving pituitary glands showed numerous and enlarged end-buds, indicating that growth had taken place. The mammary glands of rats, implanted with pituitary glands from estrogen-treated donors, however, could not be distinguished from those of rats implanted with pituitaries from normal rats. Unfortunately, Nelson[6] did not mention any comparison of the mammary glands of hypophysectomized rats receiving pituitary implants with those receiving no implants, otherwise, it is probable he would have been able to observe the direct effect of the hypophysis on the mammary gland.

The gains made in body weight of the rats receiving the two kinds of pituitary tissue contrasted markedly (Table I). There was an average gain of 17 g in body weight in the recipients of the normal pituitaries but there was an average gain of only 3 g in those receiving pituitaries from estrogen-treated donors. The amount of hypophyseal tissue implanted in the latter group exceeded that in the former because of the pituitary hypertrophy which occurs with estrogen injections. The control rats failed to gain in body weight.

TABLE I.

Group	No. of rats	Body wt, gm at beginning	Body wt, gm at autopsy	Amount of hypophyseal tissue given, mg	Adrenal wt, mg	Thyroid wt, mg	Uterine wt, mg
Controls	5	65	63	—	9.5	4.5	19.5
Injected with normal rat pituitaries	5	67	84	119	13.7	6.9	25.6
Injected with pituitaries from estrogen-treated rats	6	68	71	173	12.7	7.0	24.5

While it has been known for some time that adequate doses of estrogens will inhibit growth in the rat,[7] the results here indicate that the "hypophyseal growth factor" had been depleted from the estrogen-treated animals. This was determined by direct assay in hypophysectomized animals. The thyroid and adrenal weights of the 2 groups of rats receiving implants were not significantly different from each other but were heavier than those of the controls.

Conclusion. It is possible to demonstrate an "hypophyseal factor" in rats of both sexes which is capable of inducing growth of the mammary glands in castrated hypophysectomized immature female rats. The administration of estrogens as indicated above, does not influence this "mammogenic factor" when tested in hypophysectomized rats. The estrogen treatment was sufficient to lower the growth-stimulating power of the hypophyses when tested by direct assay.

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[7] Spencer, J., D'Amour, F. E., and Gustavson, R. G., *Am. J. Anat.*, 1932, **50**, 129.

10847 P

Bilirubin Resorption in Obstructive Jaundice.

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There is a controversy as to the mechanism of resorption of bilirubin from the liver whose common duct is obstructed. Bloom,[1] Harley,[2] and Eppinger[3] have claimed that absorption in obstructive jaundice occurs through the lymphatics of the liver, the bilirubin reaching the blood stream through the thoracic duct. Others, Whipple and King,[4] Bollman, Sheard, and Mann,[5] Mendell and Underhill[6] were as emphatic in claiming that absorption occurs through the intralobular radicles of the hepatic veins.

The following investigation was conducted with the view of throwing some light on this mechanism. Twenty-eight experiments were performed on dogs to determine the relative amounts of bilirubin absorption by the blood and lymphatics of the liver when the biliary system was placed under experimentally controlled back pressures. This was accomplished by intubating the distal end of the common duct with a cannula connected by rubber tubing to a glass reservoir. The entire system was filled with the animal's own bile which was diluted with 2 volumes of physiological saline solution. The bile in each experiment was aspirated from the gall bladder, which was excluded from the biliary tree by ligation of the cystic duct. Each experiment was conducted under a constant and definite pressure (250 to 750 mm of H_2O) by raising the bile-containing reservoir to a measured height above the level of the liver. Quantitative van den Bergh estimations of bilirubin were made on the blood and lymph at 10- to 20-minute intervals during the course of the experiment. Lymph was obtained for bilirubin determination by cannulation of the cervical portion of the thoracic duct which was severed from its entrance into the subclavian vein. The blood was

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* The authors gratefully acknowledge the valuable help and advice of Professors Wright and CoTui as well as the technical assistance of M. Lowenthal.

This work was supported by a grant-in-aid, Society of the Sigma Xi.

[1] Bloom W., *Bull. Johns Hop. Hosp.*, 1923, **34**, 316.

[2] Harley, U., *Arch. f. Anat. u. Physiol.*, 1893, p. 291.

[3] Eppinger, H., *Beitr. z. Path. Anat. u. z. Allg. Path.*, 1902, **31**, 230.

[4] Whipple and King, *J. Exp. Med.*, 1911, **13**, 115.

[5] Bollman, Sheard, and Mann, *Coll. Pap. Mayo Clin.*, 1926, **18**, 360.

[6] Mendell and Underhill, *Am. J. Physiol.*, 1905, **14**, 252.

taken from the femoral vein. The anesthetic used was pentobarbital sodium (nembutal) intravenously, 70 mg per kilo.

The experimentally produced intrabiliary pressures can be classified in 3 groups. (1) 250 mm H_2O pressure or less. In all 5 experiments the hydrostatic pressure was less than the secretory pressure of the liver as reported by Herring and Simpson[7] and subsequent investigators. (2) 300 mm H_2O pressure. In all 6 cases in this group, the intrabiliary pressure approximately equalled the secretory pressure of the liver. (3) 400 mm H_2O pressure or more. Such hydrostatic pressures were always greater than the secretory pressure of the liver.

In the first group, the experiments were done at a pressure of 250 mm of water. The liver secreted against this sub-secretory pressure and added to the reservoir from 5-12 cc of bile. At the end of a 3-hour experimental period, quantitative estimations of bilirubin in the blood and lymph were negative except for an occasional very faint trace of bilirubin in the lymph.

In the second group, the intrabiliary pressure was maintained at 300 mm H_2O. Of the 6 experiments, 3 showed bilirubin in the lymph and none in the blood throughout the duration of the 3-hour period. In the other 3 experiments, bilirubin appeared in the blood as well as in the lymph. However, the bilirubin always appeared first in greater concentration in the lymph and was later detectable in the blood, usually after 15 minutes. (Table I.)

TABLE I.

Bilirubin Concentration in the Blood and Lymph at an Intrabiliary Pressure of 300 mm of Water.

Exp.	Mg bilirubin per 100 cc	Minutes							
		10	20	30	60	80	100	120	140
A	Blood	.0	.0	.0	.0	.0	.0	.0	.0
	Lymph	.08	.17	.20	.36	.40	.90	1.30	1.60
B	Blood	.12	.12	.15	.38	.41	.40	.38	.40
	Lymph	.20	.30	.58	1.00	.98	1.20	1.50	1.60
C	Blood	.0	.05	.08	.10	.22	.30	.40	.55
	Lymph	.20	.40	.70	1.50	2.30	3.10	3.40	2.90
D	Blood	.0	.0	.0	.0	.0	.20	.30	.30
	Lymph	.0	.20	.30	1.20	1.30	1.50	1.60	1.90
E	Blood	.0	.10	.25	.40	.45	.45	.50	.55
	Lymph	.0	.20	.40	2.00	3.45	4.20	3.60	3.30
F	Blood	.0	.0	.0	.0	.0	.0	.0	.0
	Lymph	.0	.0	.0	.60	1.00	2.20	3.40	—

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[7] Herring, P. T., and Simpson, S., *Proc. Roy. Soc. Lond.*, 1907, **79**, 517.

The experiments in the third group were conducted under constant pressures of 400, 500, 600, or 750 mm respectively. The average loss of bile from the reservoir was always more than 8 cc. Under these conditions, bilirubin was present in both the blood and the lymph. It is an interesting fact that while the bilirubinemia resulting from obstructive absorption tended to attain a common level irrespective of the degree of biliary pressure, the concentration of bilirubin in the lymph appeared to vary with the degree of intrabiliary pressure. (Table II.) The mechanism underlying this phenomenon is not yet clear.

Summary. Bilirubin did not appear in the blood or thoracic duct lymph when the intrabiliary pressure was lower than the secretory pressure of the liver. Obstruction of the common duct by raising the intrabiliary pressure above the secretory pressure of the liver resulted in bilirubinemia of approximately constant level, and in the appearance of bilirubin in the lymph in concentrations which increased as the intrabiliary pressure was increased.

TABLE II.
Typical Experiments Showing Bilirubin Concentration in the Blood and Lymph at Various Intrabiliary Pressures.

Pressure, mm of water	Mg bilirubin per 100 cc	Minutes							
		10	20	30	60	80	100	140	180
200	Blood	—	.0	—	.0	.0	.0	.0	—
	Lymph	—	.0	—	.0	.0	.0	.0	—
250	Blood	—	.0	—	—	—	.0	.0	.0
	Lymph	—	.0	—	—	—	.16	.21	.19
300	Blood	.0	.0	.0	.0	.0	.0	.0	—
	Lymph	.08	.17	.20	.36	.40	.90	1.60	—
400	Blood	.0	.50	.70	.56	.44	.52	—	—
	Lymph	.0	.37	.75	1.12	1.00	1.62	—	—
500	Blood	.58	.79	.63	1.40	1.60	—	—	—
	Lymph	1.80	3.00	3.00	3.30	3.30	—	—	—
750	Blood	.58	—	.63	.63	.55	—	.75	1.16
	Lymph	3.00	—	3.50	3.75	3.25	—	3.00	3.25

10848 P

The Central Nervous System Stimulant Effects of Dextro-Amphetamine Sulfate.

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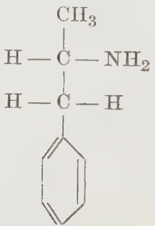
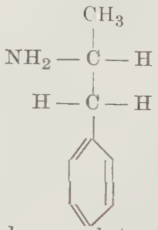
Amphetamine (Benzedrine) contains in its structure one carbon atom which is asymmetric and it has been resolved by suitable means into 2 optically active forms:


dextro-amphetamine


levo-amphetamine

A study of the comparative physiological actions of these isomers in experimental animals and in 3 normal persons, by Alles,[1] showed that the peripheral actions of these isomers are closely the same and similar to those of the racemic compound. However, the central actions of these compounds were found to differ considerably, the dextro isomer being more active than the racemic compound and from 2 to 4 times more active than the levo isomer. Trevan[2] has reported the analeptic effect against paraldehyde anesthesia of mice to be greater for the dextro isomer than for the levo isomer.

These observations made it of interest to determine whether dextro-amphetamine (dextro-Benzedrine) would prove to be more efficient clinically than either the racemic compound or its levo isomer. The clinical conditions chosen for study were those which have been found to be benefited principally by the central nervous system stimulant effects of the racemic compound (Benzedrine). The method of study in narcolepsy was similar to that of Prinzmetal and Bloomberg,[3] and in post-encephalitic Parkinson's disease like that of Solomon, Mitchell and Prinzmetal.[4] Four cases of narcolepsy were each under observation for several months, until at least 2 sets of observations of the effective dosage of the optically active isomers and of the racemic compound were completed. Two cases of post-encephalitic Parkinson's disease have also been under observation for several months, and while it is more difficult to quantitatively evaluate therapy in this disease than in narcolepsy, the relative effectiveness of the isomers is clearly apparent. One case of postural hypotension has also been studied with the isomeric compounds. In all cases studied the method was to determine a minimal effective dosage of each compound that produced comparable degrees of effect.

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[1] Alles, G. A., *Univ. Calif. Publ. Pharmacol.*, 1939, **1**, 129.

[2] Trevan, J. W., *Proc. Roy. Soc. Med.*, 1939, **32**, 391.

[3] Prinzmetal, M., and Bloomberg, W., *J. A. M. A.*, 1935, **105**, 2051.

[4] Solomon, P., Mitchell, R. S., and Prinzmetal, M., *J. A. M. A.*, 1937, **108**, 1765.

Dextro-amphetamine was found to be much more active than the levo isomer in all the cases studied. The ratio of their relative activities varied from 2 to 4. It was likewise found that the dextro isomer was more active than the racemic compound, which in turn was more active than the levo isomer. Theoretically, if the levo : dextro ratio is as 1 : 4 for their activities, the corresponding racemic : dextro ratio should be as 5 : 8. Our observations indicated that the activity of the dextro isomer was indeed from about 1.5 to 2 times that of the racemic compound.

Some supplementary observations† have been carried out on 10 normal individuals with regard to the comparative effects of the dextro and the racemic compounds on alertness and mood. These observations naturally have a low degree of precision and depend upon subjective impressions, but the dextro isomer was very clearly the more active.

10849

Electroencephalographic Studies in Relatives of Epileptics.*

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(Introduced by R. J. Block.)

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In studying heredity in nervous and mental diseases it has proved useful to study not only the incidence of identical clinical entities in other members of the family but also to observe the occurrence of other personality or physical trends, which appear to be related in some way to the disease under study. Such characteristics may be found in many members of the family who may, nevertheless, not manifest clinical evidence of the disease itself. In studies of heredity it is obviously important not only to study the direct line of descent with regard to the factors under analysis but also the total family including siblings where the latent element may possibly occur.

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† These observations by Dr. Harvey Lewis.

* This study was aided by a grant to Dr. S. E. Barrera from the Child Neurology Research (Friedsam Foundation).

The development of electroencephalography has revealed that abnormal electroencephalograms can be found in epileptics not only during seizures but also in many cases between seizures.[1, 2, 3] Considering the findings in the electroencephalogram as possible evidence of either the manifest or latent existence of an epileptic tendency it seemed important to study to what extent electroencephalographic abnormalities are present in the relatives of known epileptics.

Methods. Electroencephalographic studies were performed on all the relatives possible to contact, of epileptics committed to one of the large state hospitals of New York and diagnosed as "idiopathic epilepsy." The relatives included the parents, when possible, and all the siblings possible. A short history was taken on every relative with special reference to the incidence of nervous and mental disease and particularly of convulsive and epileptoid trends. Whenever some organic disease involving the nervous system seemed probable in a relative a complete neurological examination was performed. To date, 93 relatives of 31 epileptics have been examined.

A 2-channel electroencephalograph with ink writing recorders, constructed by one of us (W.E.R., Jr.), was used. Standard records were taken with 2 pairs of electrodes placed symmetrically in both frontal and occipital regions so that we obtained trans-frontal, trans-occipital and right and left fronto-occipital records. Patients were lying down at rest, in a darkened room, with the eyes closed.

Observations. Three types of electroencephalograms (EEG) were differentiated. These were called normal, abnormal and suspicious records. Under the classification of abnormal were included those which showed serial activity of 3 or 6 or between 3 and 6 cycles per second of an amplitude of at least 50 microvolts. In the classification of suspicious records were included those showing either similar series of lower amplitude or similar slow frequencies in relatively large numbers but without forming series. Normal records were considered as those showing none of these findings. The appearance of series of slow potentials exclusively after hyperventilation was considered as abnormal only when it occurred in adults.

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[1] Gibbs, F. A., Davis, H., Lennox, W. G., *Arch. Neurol. Psychiat.*, 1935, **36**, 1133.

[2] Gibbs, F. A., Gibbs, E. L., and Lennox, W. G., *Brain*, 1937, **60**, 377.

[3] Jasper, H. H., and Nichols, I. C., *Am. J. Psychiat.*, 1938, **94**, 853.

As stated above, our preliminary study to date includes 93 relatives of 31 epileptics. The number of relatives studied varied from 1-9 in the various families. Table I summarizes these results. Twenty-five members constituting 26.9% of the examined relatives gave definitely abnormal records. Of the 93 relatives examined 30 were parents of the epileptic patients. Seven of these or 23.3% of such parents showed abnormal records. Of the 63 siblings of the epileptic patients 18, constituting 28.6% gave abnormal records. Of the 31 epileptics whose families were examined at least one person with an abnormal record in the family was found in 14 cases or 45%. Definite abnormality of at least one parent was present in 8 cases or 34.8% of the 23 cases in which one or both parents were examined. Abnormal records in at least one sibling were found in 11 or 46% of the 23 cases in which the siblings were examined.

TABLE I.
Incidence of Abnormal Potentials in the Relatives Studied.

Group	Case No.	Parents Found			Siblings Found		
		Normal	Suspicious	Abnormal	Normal	Suspicious	Abnormal
Normal Records	1	1			2		
	2	1					
	3	1					
	4				2		
	5	1					
	6	2			4		
	7	1			2		
	8	1					
	9	1			4		
	10	2			4		
	11	2					
	12				1		
Suspicious Records	13					1	
	14					1	
	15		1				
	16	2				1	
	17				1	1	
Abnormal Records	18			1	6	2	
	19					1	2
	20	1	1		2	1	1
	21	1				1	2
	22		1			1	2
	23	1		1	1		
	24			1			
	25	1		1		1	1
	26			1	2		1
	27						2
	28			1	1		2
	29	1		1			2
	30	1					1
	31				1		2

The records in general revealed 2 family groupings. One family group consisted of those cases in which several persons with abnormal records were found. The other group consisted of those in which the relatives did not show any evidence of electroencephalographic abnormalities. Thus it is clear that the abnormal records were not equally distributed throughout the various families.

In the first group mentioned above, namely, that in which abnormal records were found in one or several relatives of the patients, there were 14 cases or 45% of 31 families examined. Fifty-three relatives of these 14 patients were examined and abnormal records were found in 25 cases or 47.2%. Normal records were found in 19 cases or 35.8% and suspicious records were found in 9 cases or 17%. In 3 cases overt familial epilepsy was present. In a total of 10 cases or 71.5%, some other clinical symptom related to epilepsy, such as epileptoid personality, migraine, etc., was present. A possible symptomatic cause for the convulsive disorder was found in only 2 cases. In the remaining 12 cases or 85.7%, a history indicating only "idiopathic epilepsy" was obtained. It is clear from the above that one group of these patients showed a high incidence of abnormal or suspicious electroencephalographic findings in the relatives examined, associated with a high incidence of clinical "epileptoid" features in the relatives and a history suggesting idiopathic epilepsy in the patients.

The second group included those cases in the relatives of which no abnormal electroencephalographic patterns were found. All that can be said at present is that such abnormal potentials were not found even with hyperventilation. Since abnormal potentials are not always present and cannot always be produced by hyperventilation, even in overt epileptics, it is possible that abnormal records in some of the examined subjects were missed. In some cases, in addition, it was not possible to obtain records on all relatives of the patient. Thus, it is again possible that some of those relatives were missed who might have shown abnormal records if recorded. Preliminary observations, however, to date indicate the probability of the existence of these 2 groups of relatives and final answer can be obtained only as the study is continued in a large number of cases. It is of interest that this group of 12 patients in which 32 relatives were examined with no abnormal electroencephalographic findings, showed clinical epileptoid characteristics in only 4 cases or 33⅓% of the relatives and a history very probable of symptomatic epilepsy in 6 cases or 50%.

Although, as mentioned above, the relatives examined tended to separate themselves out on the basis of their electroencephalographic records into a group with a high incidence of abnormal records and a group with no abnormal records, there was a small third group of 5 epileptics with 8 relatives in which no definitely abnormal but some suspicious records were found. Of the 8 examined relatives in this small group normal records were found in 3 cases or 37.6% and the suspicious records in 5 cases or 62.5%. Clinically epileptoid trends in the family were found in 3 cases or 60% of these 5 families and a history of "idiopathic epilepsy" in all of the cases. Although we feel that ultimately the suspicious records will have to be included in the group of pathological or abnormal records these records are being kept for the present in a separate group until the problem is more definitely controlled on a largr series of cases.

Discussion. As a result of the preliminary studies made so far it is evident that many subjects with abnormalities of the electroencephalogram can be found in the families of some epileptics and also that similar abnormalities are not found in the families of other epileptics. On the basis of these 2 groups of electroencephalographic findings it seems possible that in time, on the basis of the electroencephalogram, at least 2 forms of epilepsy may be established; one with abnormal brain potentials in the family and one without abnormal brain potentials in the family. It is highly probable that many cases now diagnosed as "idiopathic epilepsy" in our hospitals are not definite idiopathic epileptics but are related to some more specific, as yet undetermined causes, which would place them in the group of symptomatic convulsive disorders. Further studies are being made to determine whether these 2 groups of patients, as determined by the incidence of abnormal electroencephalographic findings in their families, correspond to the 2 groups which on careful clinical examination might be separated out into the idiopathic and the symptomatic convulsive disorders.

A still more important question arising from these findings, certainly from the practical genetic or therapeutic angles, is the question as to whether relatives, clinically non-epileptic, showing abnormal electrical patterns may develop overt seizures at some time or other. This would seem to be especially important in those young siblings of epileptics, in which the abnormal electroencephalographic findings are present even though overt seizures have not occurred. It seems highly probable that only a small percentage of relatives with abnormal electroencephalographic findings ever develop overt clinical seizures. A long study and follow-up will have to determine whether those cases which will, at some time or other, develop overt seizures can be separated out from the other relatives who, although they manifest abnormal electroencephalographic findings, fail to develop overt seizures. In addition, another important question is whether such cases can be detected in early childhood and preventive measures of some sort taken to eliminate the probability of the onset of overt seizures. Finally, the problem as to the "carrier" nature of such relatives with regard to the heredity of convulsive disorders is a highly important one on which it is hoped the long controlled series of observations now under way at the New York State Psychiatric Institute may shed future light.

Summary. Upon examination of parents and siblings of patients diagnosed as idiopathic epileptics it was shown that 23% of the parents and 28% of the siblings showed definitely abnormal EEG findings. The material fell roughly into 2 groups, that is, those families showing a high incidence of abnormal EEG findings and those families showing no such evidence. The diagnostic, therapeutic and genetic problems are briefly reviewed in relation to these observations.

10850 P

Fluorescence and Absorption Spectra of Flavin Isolated from a Toxic Culture Filtrate of *C. diphtheriae.*

M. O'L. CROWE. (Introduced by Augustus B. Wadsworth.)

From the Division of Laboratories and Research, New York State Department of Health, Albany.

This note* reports the isolation of flavin elaborated by *C. diphtheriae,* strain No. 3203, grown in synthetic medium. Flavin was separated by ultrafiltration from the toxin and extracted by chloroform from the filtrate after it had been treated with ether to remove the porphyrins soluble in ether and any other ether-soluble substances. The chloroform extract was subjected to chromatographic analysis and the pigment solutions that were recovered from the column were centrifugalized.

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* This study is a continuation of previous studies[1,2,3] of toxigenic growth of the diphtheria bacillus in synthetic medium. I wish to thank Miss Amy Walker for her collaboration in the technical work.

[1] Wadsworth, Augustus, Crowe, M. O'L., and Smith, L. A., *Brit. J. Exp. Path.*, 1935, **16**, 201.

[2] Wheeler, M. W., and Crowe, M. O'L., *J. Bact.*, 1936, **31**, 519.

[3] Crowe, M. O'L., PROC. SOC. EXP. BIOL. AND MED., 1937, **37**, 215.

The green fluorescence of the untreated filtrate suggests the presence of flavin. Others[4, 5] have also noted this fluorescence. The chloroform extract was analyzed on a column of Brockmann's standardized aluminum oxide. When the column was examined in the light of a Hanovia analyzing lamp, several fluorescing zones were observed. The material in the lowest zone, of a brilliant canary yellow fluorescence, was washed from the column with chloroform and, in this solution, fluoresced with a yellow-green light that changed to blue when the solution was exposed to daylight. The material in

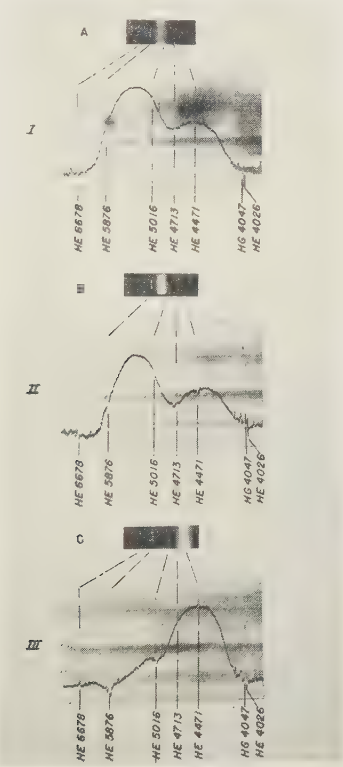

FIG. 1.

Fluorescence spectrograms, A, B, C, of the chloroform extract of an ether-extracted residue of an ultrafiltered diphtheria toxin filtrate; and of the 2 pigments in this extract recovered from a Tswett column in chloroform and methyl alcohol solutions, respectively; and the microphotometric tracings, I, II, and III, of these spectrograms.

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[4] Dhéré, Ch., Meunier, P., and Castelli, V., *C. r. soc. de biol.*, 1938, **127**, 564.

[5] Dhéré, Ch., and Gourévitch, A., *C. r. soc. de biol.*, 1939, **130**, 593.

the zone above this, which fluoresced with a bright blue light, was elutriated from the column with methyl alcohol and fluoresced in this solution with an intense sky blue light which changed to yellow when the solution was made alkaline and remained so when left standing in daylight. The addition of a reducing agent, sodium hydrosulfite, quenched the fluorescence which was restored when the solution was oxidized by shaking in air.

Uninoculated synthetic medium, when poured on the colunmn, gave a very feeble fluorescence not at all similar to the brilliant yellow and blue light described above.

The fluorescence spectrum of the pigment component of Warburg's yellow enzyme has been reported[6] as beginning at λ 500mμ and ending indefinitely at λ 630mμ. The fluorescence spectrograms of the chloroform extract and the chloroform and methanol solutions recovered from the column, Fig. 1, A, B, and C, respectively, and their microphotometric tracings, I, II, and III, indicate bands in the same general region between the mercury and helium lines λ 4047 Å and λ 6678 Å, respectively.

While the general contour of the absorption spectrum of the methanol solution recorded on an Eastman panchromatic plate is similar to the curves published for the photoderivative of the pigment component of the yellow enzyme by Warburg and Christian,[6]

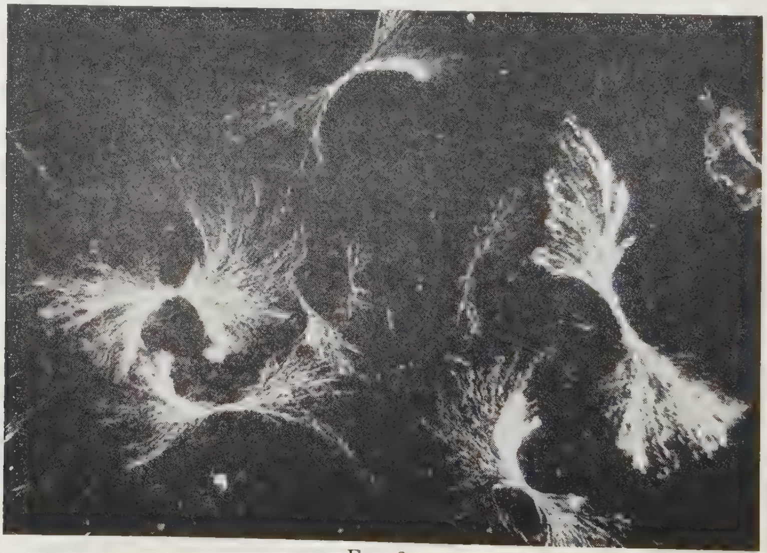

FIG. 2.

Photomicrograph of crystals of the photoderivative of the pigment isolated from diphtheria culture filtrate.

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[6] Warburg, Otto, and Christian, Walter, *Biochem. Z.*, 1933, **266**, 377.

the wave lengths of the positions of selective absorption fall in most instances further towards the violet end of the spectrum at approximately λ 250 mμ, 335 mμ, 370 to 380 mμ, and λ 460 mμ.

The pigment in the concentrations studied is optically inactive or nearly so.†

Crystals were obtained from a water solution of the pigment obtained from the methanol solution. The photomicrograph of the tufts of fine needle-like crystals is presented in Fig. 2 and resembles that of the photoderivative of the pigment component of the yellow enzyme.[6]

Since the tufted arrangement of the crystals in Fig. 2 is somewhat different from that of the photoderivative of the pigment component of Warburg's yellow enzyme, and since the absorption spectrum of the methanol solution of pigment isolated from diphtheria filtrate indicates selective absorption at slightly different wave length values than those published for flavins, it is possible that the flavin synthesized by the diphtheria organism is an unidentified flavin.

10851 P

Experiments on the Mechanism of Gastrulation in a Frog's Egg.

A. M. SCHECHTMAN AND L. E. SMALE.

From the Department of Zoology, University of California at Los Angeles.

In the experiments here reported we have attempted to analyze the factors responsible for the turning in (involution) of the dorsal lip of the blastopore. Spemann[1] states that the dorsal lip possesses an inherent capacity to turn in, although under certain abnormal conditions it may fail to do so, growing out into a horn-like projection. Vintemberger[2, 3] found that the chordal material has a weaker capacity to turn in than the prechordal portion, which lies just below the former. The chordal material is, according to this author, aided in its involution by its attachment to the prechordal material.

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† The small quantity of material available for this study did not permit the estimation of the quantity of flavin in the toxic filtrate. Further details will be presented in a report to be published elsewhere.

[1] Spemann, H., *Embryonic Development and Induction,* 1938, p. 105, Yale University Press.

[2] Vintemberger, P., *Compt. Rend. Soc. de Biol.,* 1938, **127**, 435.

[3] *Ibid.,* **127**, 436.

In the present experiments the early gastrulae of *Hyla regilla* were used. In a first series of experiments various portions of the dorsal lip were transplanted into the roof (presumptive ectoderm) of other early gastrulae (Fig. 1). The material immediately around the blastopore (circum-blastoporal material, Fig. 1) invariably invaginates to form a deep archenteric sac. However, *the mesoderm above this region* (external mesoderm, Fig. 1) *has little or no capacity to involute, whether it is transplanted alone or in combination with the internal mesoderm or the circumblastoporal material.* Under the above conditions the chordal material (present in external mesoderm, Fig. 1) grows out into a horn-like projection. Our results are apparently contradictory to Vintemberger's, who found that the presence of the prechordal material aids the chordal material in its turning in. In our work with *Hyla* we wish to emphasize that the chordal material did not turn in, in spite of its attachment to the prechordal region or to the internal mesoderm.

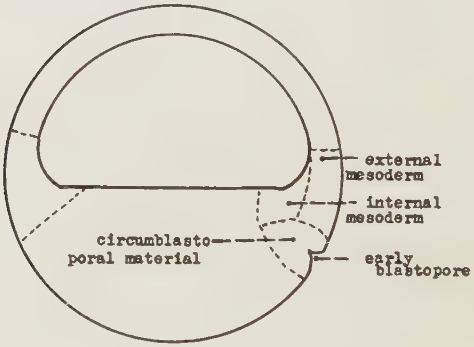

FIG. I

Showing parts of early gastrula transplanted in experiment one.

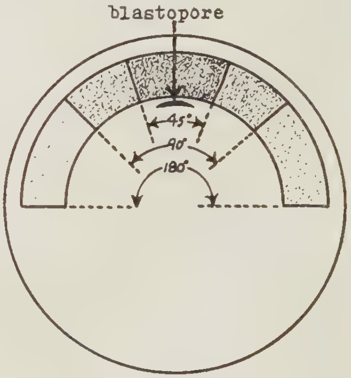

FIG. II

Ventral view showing parts of early gastrula transplanted in experiment two.

What, then, are the conditions necessary to bring about the normal involution of the mesoderm? An answer to this question is suggested by a second series of experiments indicated in Fig. 2, in which only the external mesoderm was transplanted. The smallest transplants (45°, Fig. 2) failed to involute, growing out into fingerlike extensions. Wider transplants (90°, Fig. 2) also developed projections, but these were clearly shorter than the above. The widest transplants (180°, Fig. 2) formed only minute projections. The greater part of the transplant turned under and induced a secondary neural plate almost equal to the host's in size. These results corroborate the statements of Spemann[1] and Vintemberger[2] that a transplanted dorsal lip involutes better in the marginal (mesodermal) region than in the roof of the egg. These results emphasize further that the successful involution of the dorsal lip depends specifically upon its attachment to laterally located mesoderm, rather than upon its relationship to the egg as a whole. Thus, a dorsal lip will involute well even when placed into the roof (ectoderm) of the egg, provided however that it is accompanied by a sufficient quantity of laterally located mesoderm.

Summary. Our work with *Hyla regilla* shows that the mesoderm of the dorsal lip of the blastopore has little or no inherent capacity for involution. The material immediately around the early blastopore (the pharyngeal endoderm and prechordal mesoderm), although itself having a strong capacity for involution, does not bring about the involution of the mesoderm above it. On the other hand, *excellent involution of the dorsal lip mesoderm occurs when it is transplanted together with laterally located mesoderm.*

10852 P

Vaccination of Monkeys with Agar-Tissue Cultures of European Rickettsiae.

Florence K. FitzPatrick. (Introduced by H. Zinsser.)

From the Department of Bacteriology and Immunology, Harvard University Medical School.

In a previous paper, we reported the successful vaccination of guinea pigs against European typhus, using agar-slant tissue cultures.[1] Since guinea pigs do not give a Weil-Felix reaction, and since they show very little other than a temperature during the course of a typhus infection, it seemed desirable to test our vaccine on animals which more nearly resemble man in their reactions. Three *Macacus rhesus* monkeys of approximately the same size were selected. One was reserved as a control, the other 2 were vaccinated with formolized vaccine prepared in the following manner: Rickettsiae of classical typhus were grown with minced mouse embryo tissue on agar slants for 7 to 9 days. The tissue was removed from the slants and ground in a small mortar, after which 3 cc of 0.2% formolized saline were added for each slant. (For human use, we recommend a light centrifugation at this point.) After being cultured for sterility, the vaccine was allowed to stand in the ice box for a week before use to insure the death of the rickettsiae. The vaccine was administered subcutaneously at 5-day intervals. Monkey No. 1 was given a total of 4 cc, the equivalent of 1⅓ agar slant cultures, the amount used in the guinea pig experiments previously reported. Two weeks after the last dose, the Weil-Felix reaction was negative, the agglutination for European Rickettsiae 1/20++, 1/40++, 1/80++, 1/160+. Monkey No. 2 was given a total of 6 cc of vaccine, representing 2 agar slant cultures, in doses of 1, 1, 2, and 2 cc. Three weeks after the last dose this monkey's serum showed an agglutination with *B. proteus* 0 x 19, of 1/20 and a Rickettsia agglutination of 1/20+++, 1/40+++, 1/80++, 1/160++, 1/320+. No local or general reactions were observed in the animals during vaccination. The test for immunity was performed 6 weeks after vaccination on monkey No. 1, 3 weeks after on monkey No. 2. They received 4 cc of virulent passage blood intraperitoneally, a normal monkey, No. 3, serving as control. The vaccinated monkeys maintained their good appetites and remained well. The control developed fever and loss of appetite on the 7th day, these symptoms persisting for 7 days. Sixteen days after inoculation, this animal had a Weil-Felix of 1/640 and a rickettsia agglutination of 1/1280. There was no change in the titer of agglutinins in the vaccinated monkeys. Brain sections of the control made on the sixteenth day showed very few lesions, giving the impression that the brain had been removed too late for pathological study.

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[1] Zinsser, H., FitzPatrick, F., and Wei, H., *J. Exp. Med.*, 1939, **69**, 179.

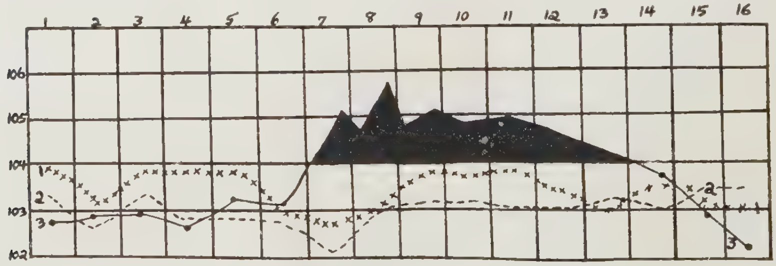

CHART 1.

Temperature curves of vaccinated monkeys (1 and 2) and of control monkey (3) after receiving 4 cc of European passage blood intraperitoneally. (Black area indicates significant temperature elevation.)

10853 P

Vaccination Against Spotted Fever with Agar-Tissue Cultures.

FLORENCE K. FITZPATRICK. (Introduced by H. Zinsser.)

From the Department of Bacteriology and Immunology, Harvard University Medical School.

The making of vaccines against the Rickettsial diseases with the contents of infected insects offers many technical difficulties to say nothing of the danger to those involved. There is no doubt that vaccines prepared in this manner by Weigl for European typhus, and by Parker for spotted fever, give considerable immunity.[1, 2] To eliminate the danger and expense of manufacture, culture vaccines of rickettsiae are the ideal, but until recently it has not been possible to obtain cultures that were sufficiently rich to permit vaccination on a large scale. By means of the agar slant method previously reported, we have been able to make vaccine which protects guinea pigs[3] and monkeys[4] against large doses of European typhus. It was hoped that spotted fever vaccine made in the same manner would be equally efficient.

Culture strains of spotted fever (Eastern type) are easily initiated with spleens of infected guinea pigs.[5] After the first generation, mouse embryo tissue is used. For the preparation of vaccine, the infected material from slants is ground in a mortar, and 3 cc of

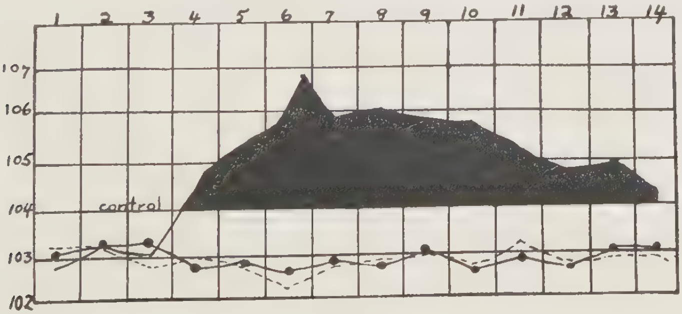

CHART 1.

Temperature curves of 2 vaccinated pigs and of control after receiving 1 cc of spotted fever blood intraperitoneally. (Black area indicates significant temperature elevation.)

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[1] Nicolle, C., and Sparrow, H., *Arch. Inst. Past. Tunis*, 1933, **21**, 25.

[2] Parker, R. R., *J. Inf. Dis.*, 1935, **57**, 78.

[3] Zinsser, H., FitzPatrick, F., and Wei, H., *J. Exp. Med.*, 1939, **69**, 179.

[4] FitzPatrick, F., PROC. SOC. EXP. BIOL. AND MED., in press.

[5] FitzPatrick, F., PROC. SOC. EXP. BIOL. AND MED., 1938, **39**, 501.

formol-saline is added for each slant. In vaccinating guinea pigs, a total of 4 cc of vaccine is administered subcutaneously in doses of one, one, and 2 cc at 5-day intervals. Animals so treated are found to be solidly immune when tested a month later with blood virus (Chart 1). Serum from 2 vaccinated pigs taken 3 weeks after vaccination agglutinated spotted fever rickettsiae (chick tissue slants) in dilution of 1/20+++, 1/40++, 1/80+. These 2 sera also protected normal guinea pigs when 1 cc was mixed with passage blood and injected after standing 40-60 minutes at room temperature.

10854

Estrogenic Properties of Extracts of Ovaries of Certain Marine Invertebrates.*

J. KENNETH DONAHUE. (Introduced by W. W. Swingle.)

From the Department of Biology, The College of Charleston, and the Bermuda Biological Station for Research, Inc.

The writer has previously reported that extracts of the ovaries of a Bermuda echinoderm *(Lytechinus variegatus)* caused considerable growth of the epithelium of the uterus and vagina when injected into the mature, ovariectomized rat.[1] However, failure of the uterus to swell with fluid as it does in full normal estrus indicated that the response was subthreshold and, possibly, that the stimulating agent in the extracts differed in some respects from estrogens derived from mammalian sources. The present study was undertaken in order to test for estrogens in other invertebrate ovaries and to make further observations of the effects of the ovarian extracts upon the vaginal and uterine epithelium of the rat.

The ovaries of the common sea urchin *(Lytechinus variegatus)*, the reef urchin (Echinometria), the holothurian *(Stichopus mobii)*, and the lobster *(Palinurus argus)* were collected in Bermuda during the summer of 1938 in considerable quantities. In all cases the ovaries were sexually mature or were rapidly approaching that state when collected. After removal the ovaries were drained of as much sea water as possible and then thoroughly ground in a mortar. The ground tissues were then placed in separate containers of 95%

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* The expenses of this investigation were defrayed in part by a grant from the Penrose Fund of the American Philosophical Society.

[1] Donahue, J. K., and Jennings, E. D., *Endocrinology*, 1937, **21**, 5.

alcohol. Extraction work was carried out in Charleston the following autumn and winter. The ovarian material was extracted 3 times with 5 times its volume of 95% ethyl alcohol. Each of the extraction periods lasted for 48 hours during which time the mixture was shaken frequently. The filtrates were combined and concentrated by distillation under reduced pressure. The tissue residues were extracted in a similar manner with acetone and petroleum ether and concentrated. The heavy, oily concentrates were combined and used either in this form or mixed with various dilutions of corn oil. This procedure was carried out for all of the species employed. All extracts, with the exception of the one obtained from Palinurus, gave positive Salkowski reactions indicating the presence of sterols. In the dosages employed the extracts were nontoxic.

Mature, ovariectomized rats of the Wistar strain were used as test animals. Following ovariectomy they were left untreated for 10 days. With the previous knowledge that such extracts contain, at best, but small quantities of estrogens several modes of administration were adopted in order to ascertain that which would give a maximal response. In some cases subcutaneous injections were given throughout the standard 10-day injection period (designated "s.c" in Table I). In others subcutaneous injections were given every other day and direct applications of extract into the vagina made on alternate days (designated "s.c.-v." in Table I). Finally, some of the animals received direct applications of extract into the vagina throughout the period of treatment (designated "v." in Table I). All animals were killed on the 11th day and the uteri and vaginæ

TABLE I.
Effect of Invertebrate Estrogens on the Vagina and Uterus of the Rat.

Rat and source of estrogen	Age in days	Daily dosage		Route	Effect on vagina*	Effect on uterus	Injection period, days
		extract, cc	corn oil, cc				
S 1	190	.25	.25	s.c.	none	none	10
S 2	190	.4	.1	s.c.-v.	12	hyperemic	10
S 3	80	.25	.25	v.	8	"	10
S 4	80	.4	.1	v.	9	"	10
E 1	190	.25	.25	s.c.	3	slight	10
E 2	190	.4	.1	s.c.-v.	12	hyperemic	10
E 3	90	.25	.25	v.	5	"	10
P 1	190	.25	.25	s.c.-v.	4	slight	10
P 2	90	.25	.25	v.	5	"	10
L 1†	190	.25	.25	v.	0	none	10

"S"—Stichopus, "E"—Echinometria, "P"—Palinurus, "L"—Lytechinus.

* Figures indicate approximate number of newly grown cell layers in vaginal epithelium.

† This extract 1½ years old.

fixed for histological study. Vaginal smears were taken once daily during the entire experimental period, great care being exercised not to traumatize the vagina. When the extract was applied directly into the vagina the animal was held head downward and the extract gently forced into the vagina with a pipette. The results of this procedure are summarized in Table I.

The smear records followed fairly identical courses in those cases in which positive reactions occurred. After the third day of treatment a marked diminution of leucocytes and the appearance of large numbers of nucleated epithelial cells and cornified epithelial cells was noted. In 3 cases full estrous smears appeared on the 7th or 8th day (E2, P1, and S2). In the majority of cases the reappearance of leucocytes around the 10th day was noted, indicating that the establishment of a growth phase in the vaginal epithelium was not as permanent as is the case when standard estrogens are given. As the table indicates, the uteri of the positively reacting animals showed some hyperemia but no gross signs of swelling with fluid even in those instances where full estrous smears were obtained. For controls 3 ovariectomized rats were injected with 0.5 cc of corn oil daily for 10 days; 3 received alternate injections and vaginal applications of the same amount of corn oil daily for 10 days; and 3 received vaginal applications of corn oil daily for 10 days. In no case did this treatment disturb the diestrous phase resulting from ovariectomy. At this point quantitative data include only the yield of crude extract from equivalent amounts of ovarian tissue in the dry state. Ninety cc of Stichopus extract represented 90 g of dried ovarian tissue. Two hundred cc of Echinometria extract represented 55.5 g of tissue and 49 cc of Palinurus extract represented 25.5 g of tissue.

It is concluded that all of the extracts tested contained estrogenic material. Unfortunately injections of this type of extract amounting to more than 0.5 cc per day are not well tolerated. It is likely that higher dosages would have given full estrous reactions in a larger number of animals. It appears from these results that estrogenic materials from this source have a specific effect upon cell growth of the vaginal epithelium and, to a lesser extent, upon the epithelium of the uterus. However, they do not effect the fluid shift which usually occurs into the lumen of the uterus in estrous produced in other ways. The suggestion is made that estrogenic substances from this source may not contain the chemical complex responsible for the uterine edema found in normal estrous rats.

10855 P

Mechanism of Estrogen Effect on Nasal Mucosa in Atrophic Rhinitis; Successful Treatment with Prostigmin.

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From the Department of Metabolism and Endocrinology, and Department of Otolaryngology, Michael Reese Hospital, Chicago, Illinois.

Periodic changes in the appearance of the nasal mucosa during the menstrual cycle, have long been recognized. A consideration of this so-called "naso-sexual relationship" led Mortimer, Wright and Collip[1] to observe the effects of parenterally administered estrogenic substance upon the mucous membranes of the nose. The changes which they observed suggested the use of estrogens in the treatment of atrophic rhinitis. They reported clinical improvement in such cases, following the repeated application of oily solutions of estrone directly to the nasal mucous membranes. Blaisdell[2] arrived at the same therapeutic idea from the similarity in the pathology of atrophic rhinitis and of atrophic or senile vaginitis, and from the previously reported efficacy of local estrogen therapy in the latter condition. He also reported clinical improvement in atrophic rhinitis obtained by intranasal estrin therapy.

The local treatment of atrophic rhinitis with estrone has produced only mildly favorable or negative results in our hands. We were also impressed by the lack of any evidence of gonadal deficiency in most of our cases. Both these facts deprecated the importance of the "naso-sexual relationship", but did not offer any other explanation for such therapeutic results as were obtained with the estrogens. The situation was clarified by a recent study of one of us and coworkers,[3] in which it was found that the various component phenomena of estrus may be classified into 2 categories:

1. The specific proliferative effects of estrin, and
2. Those effects which are secondary to hyperemia.

The latter effects can be reproduced by non-specific hyperemic agents such as yohimbin. However, when they result from estrin action, they are due to the acetylcholine-liberating properties of this hormone, as demonstrated by Reynolds.[4] This suggested to us the probability that the benefit derived from intranasal estrone therapy in atrophic rhinitis depended upon the incidental hyperemic effects of the hormone, rather than upon a specific sexual relationship. It also indicated the possibility that a mode of therapy especially directed towards a physiologic hyperemia of the nasal mucosa might give better results than those obtained with estrone, and at the same time avoid unnecessary exposure to this potent hormone. It seemed logical to try the local application of acetylcholine. But since this seemed not without danger, we decided to potentiate the naturally occurring acetylcholine in the nasal mucosa, by inhibiting the choline-esterase with a synthetic physostigmine-like substance which is commercially available.

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[1] Mortimer, H., Wright, R. P., and Collip, J. B., *Can. M. A. J.*, 1937, **37**, 445.

[2] Blaisdell, I. H., *Laryngoscope*, 1938, **48**, 699.

[3] Hechter, O., Lev, M., and Soskin, S., *Endocrinology*, in press.

[4] Reynolds, S. R. M., *J. Physiol.*, 1939, **95**, 258.

To date we have treated 14 patients suffering from advanced atrophic rhinitis with ozena, for periods ranging from 2 to 6 months. Our procedure was to teach the patients to spray 0.25 cc of a 1:2000 solution of Prostigmin Methylsulfate* into each nostril, 4 times per day. To insure good contact of the medication with the mucous membrane they irrigated the nose with a mildly alkaline solution once or twice per day. All other therapy was stopped. Our results were far superior to those obtained with estrone or with any other recognized form of treatment. Without exception, all patients exhibited definite improvement within about 2 weeks, and progressed favorably thereafter. There was a decrease in crust formation and in the amount and character of the nasal discharge, the latter becoming less purulent and less tenacious. The ozena was controlled. The nasal, pharyngeal, and laryngeal mucosa lost its dry, blazed appearance and became moist and pink. Patients who had previously complained of great discomfort from pharyngitis and laryngitis sicca, were completely relieved. In a number of instances there was a definite space reduction in the nasal vaults, although not to within normal limits.

We have not, as yet, had the opportunity of observing the degree of permanence of the beneficial results when the treatment is stopped. However, the results would be eminently worthwhile even if treatment had to be continuous. We have not observed any untoward results or unpleasant side reactions from the daily use of the drug, in the specified amounts.

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* Supplied by Hoffmann-La Roche, Inc., through the courtesy of Dr. R. D. Shaner.

10856 P

Production of a Sustained Neurogenic Hypertension of Renal Origin.

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From the Department of Pharmacology, University of Ghent, Belgium.

Several methods have been devised for producing experimentally a renal ischemia hypertension. That of Goldblatt, Lynch, Hanzal and Summerville[1] is most frequently used. These methods have all involved the use of a foreign material such as a clamp and have produced blood pressure changes that are not reversible without removing the foreign material or the involved kidney.

It has therefore been difficult to apply these technics to experimental studies analysing the value of various methods of surgical treatment of hypertension in man. It has also been difficult to evaluate the possible importance of a primary nervous factor or a primary vascular disease factor in attempting to correlate experimental renal ischemia hypertension with essential hypertension in man.

A technic has been devised to determine in dogs whether an overactivity of the renal vasoconstrictor splanchnic nerves can produce hypertension. The technic consists of sympathectomizing all of the animal except the origin and the distribution of the splanchnic nerve fibers to the kidneys and adrenals. This is accomplished in 3 operations. At the first the abdominal sympathetic chains below the second lumbar ganglion are removed and the rami to that ganglion as well as the abdominal branches leaving the coeliac ganglion except those to the kidneys are divided. The first lumbar nerve is now cut distal to the origin of the sympathetic ramus. At the second and third operations the first 7 thoracic sympathetic ganglia, including the stellate, are removed and the last 5 intercostal nerves are divided near but distal to the origin of the sympathetic rami on both sides. This leaves the animal completely sympathectomized with the exception of the kidneys and the adrenals.

Five dogs were prepared in the hope that this extensive sympathectomy might produce an overactivity of the remaining portion of the sympathetic system; the innervation of the kidneys and adrenal glands. During periods of observations varying from 25 to 60 days, however, no alteration of the general blood pressure from that before and during the operative stages was noted. Therefore to intensify the activity of the splanchnic nerves, 3 dogs have been further operated according to the following technic:[2, 3] One cervical vagus-sympathetic-depressor nerve and the depressor-sympathetic nerve on the other side were cut, and the carotid sinuses were excised. This total removal of the reflex moderator nerves of the cardio-vascular centers produces, in normal dogs, an immediate and sustained increase of the sympathetic vasoconstrictor tone and arterial hypertension.[2–5] These reactions are absent in completely sympathectomized dogs.[3, 4, 6] In the 3 animals with the moderator nerves cut and with only the sympathetic innervation of the kidneys and the adrenals left the blood pressure rose gradually and reached a sustained hypertensive level in 5 to 14 days. The increases of blood pressure were respectively from around 100 to around 170 mm Hg in one, 130 to 200 in the second, and 135 to 225 in the third dog. The blood pressures remained elevated during 20 to 30 days of observation. Now, although the delayed gradual blood pressure elevation and the rates (120, 126 and 138 average respectively) of the sympathectomized hearts argue against the adrenals as a cause of the hypertension, a control was made in 2 animals by denervating the kidneys at their pedicle. This restored the blood pressure to normal where it remained during the following 90 and 40 days respectively of observation. The kidneys of 2 of the 3 hypertensive dogs were observed, one at operation and one at autopsy. They appeared somewhat smaller and darker than normal.

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* Fellow of the Belgian American Educational Foundation.

[1] Goldblatt, H., Lynch, J., Hanzal, R. F., and Summerville, W. W., *J. Exp. Med.*, 1934, **59**, 347.

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[2] Heymans, C., Bouckaert, J. J., and Regniers, P., *Le sinus carotidien,* 1933, O. Doin et Cie, edit., Paris.

[3] Heymans, C., and Bouckaert, J. J., *C. R. Soc. Biol.,* 1935, **120**, 82; *Summaries of Communic. XV intern. Physiol. Congr.,* 1935, p. 154; *Bull. Academ. roy., medec. belg.,* 1936, p. 42; *Acta brev. neerl.,* 1939, in press.

[4] Heymans, C., *Surgery,* 1938, **4**, 487; *New Eng. Med. J.,* 1938, **218**, 147.

[5] Nowak, S. J. G., and Walker, I. J., *New Eng. Med. J.,* 1939, **220**, 269.

[6] Bacq, Z., Brouha, L., and Heymans, C., *Arch. intern. pharmacodynam.,* 1934, **48**, 429.

10857 P

Vascular Changes in Renal Ischemia Cell Mitosis in the Media of Arteries.

N. Goormaghtigh and K. S. Grimson.

From the Department of Pathology and of Pharmacology, University of Ghent, Belgium.

Pathological studies of the kidneys of chronically hypertensive dogs with renal ischemia by Goldblatt, Lynch, Hanzal, and Summerville,[1] by Verney and Vogt,[2] and by others have in general revealed only degenerative changes chiefly of the tubules, arterioles and to a lesser extent of the glomeruli. These changes have been attributed directly to the ischemia. No changes have previously been described which might have a direct relationship to the hypertensive principle of the ischemic kidney. In order to see if such changes exist studies of the more immediate as well as the chronic effects of partially obstructing the renal arteries are being made by one of us (Goormaghtigh).

The kidneys of 12 dogs sacrificed 2 days to 18 months after compression of the renal arteries by Golblatt clamps applied at 2 consecutive lumbar operations, or by externally adjustable clamps[3] applied simultaneously have been studied. The most striking change has been observed in the media of the interlobular and glomerular arteries.

The media of these vessels is composed not only of ordinary smooth muscle cells but also of larger, more afibrillar and probably less contractile cells.[4, 5] In the kidney these cells are found all along the arterial vascular tree and accumulate in groups at the vascular poles of the glomeruli to form the "juxta glomerular apparatus" or "polkissen". Elsewhere in the body these cells are particularly abundant in the arterial vessels of the glomus carotidum and glomus aorticum[6] and in the vascular glomi of the skin. They are also found in a subendothelial position in the region of the carotid sinus.[6] Their close association with the carotid sinus and cardioaortic presso and chemo receptor regions suggests that their function may be connected with vaso-motor regulation. Masson[7] suggests that they do have a function of local vascular control.

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[1] Goldblatt, H., Lynch, J., Hanzal, R. F., and Summerville, W. W., *J. Exp. Med.*, 1934, **59**, 347.

[2] Verney, E. B., and Vogt, M., *Quart. J. Exp. Physiol.*, 1938, **28**, 253.

[3] Grimson, K. S., *J. Physiol.*, 1939, **95**, 45p.

[4] Goormaghtigh, N., *J. Physiol.*, 1937, **90**, 1263.

[5] Goormaghtigh, N., and Handovsky, H., *Arch. Path.*, 1938, **26**, 1144.

[6] Goormaghtigh, N., and Pannier, R., *Arch. Biol.*, 1939, **50**, in press.

In the media of the arteries and arterioles of the examined ischemic kidneys of hypertensive dogs these large, afibrillar and sometimes granulated or vacuolated cells have increased in number and size. In all animals the juxta glomerular apparatus is enlarged. Its cells frequently have very large nuclei and are often vacuolated. In the proximal segment of some of the intralobular arteries of some of the animals almost all of the smooth muscle cells have enlarged and become afibrillar with hypertrophied nuclei. The intensity of this type of transformation is emphasized by the presence of numerous mitotic figures. In some places these hypertrophied afibrillar cells obstructed the glomerular afferent arterioles and effect extensive atrophy of the associated glomeruli. In acutely ischemic kidneys these vascular changes are associated with varying degrees of tubular changes. In the chronically ischemic kidneys only the alteration of the afibrillar cells is consistently observed. In one animal sacrificed 18 months after application of the clamps regressive changes comparable to those observed at the vascular pole in human constricted kidneys were observed.

In general these hyperplastic cell changes stand out in contrast to the degenerative changes described by previous authors. The possibility that these cells may have a local or even general secretory or humoral activity and may therefore have a direct relationship to the hypertensive principle of the ischemic kidney must be considered.

Similar hypertrophy and hyperplasia of this type of cell has been observed in hypertensive hypervitaminose "D" dogs[5] and in the kidneys of scarlet fever patients.[8] Mitotic cell division in the media of arteries has not been reported in the literature but one of us (Goormaghtigh) has observed it in the kidneys of hypervitaminose "D" rabbits and of normal rabbits with one ureter ligated 4 days previously.

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[7] Masson, Pierre, *Les Glomus Neuro-Vasculairus*, 1937, Paris, Hermann et Cie.

[8] Goormaghtigh, N., *Arch. de Biol.*, 1932, **43**, 575.

10858 P

Nature of the Pilomotor Response to Acetylcholine.

Julius M. Coon and Stephen Rothman. (Introduced by E. M. K. Geiling.)

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Pilomotor reactions to choline esters[1-4] have been somewhat puzzling in view of the accepted concept of a single sympathetic innervation to the pilomotor muscles.

When 0.1 cc of acetylcholine in dilutions between 1:10^4 and 1:10^5 is given intracutaneously in man a goose flesh appears immediately in the area surrounding the puncture and subsides in about 2 minutes. The picture simulates closely that of the physiological pilomotor reaction and differs markedly from the goose flesh produced by intradermal epinephrine injection in concentrations of 1:1000 to 1:100,000, which produce a long lasting goose flesh confined to the vasoconstricted area.

A pilomotor response to intradermal injection of acetylcholine can also be seen in the cat's tail. Furthermore, injection of acetylcholine into the sacrocaudal artery produces a general pilo-erection on the tail. This response is not modified by heavy atropinization or eserinization of the cat. In man, however, physostigmine increases slightly the extent and doubles the duration of response if mixed in a concentration of 1:400,000 to 1:1,200,000 with effective intradermal doses of acetylcholine.

The optimum concentration of acetylcholine (1:40,000) elicits pilo-erection repeatedly if inserted at the same point in the human skin or cat's tail. But if preceded by an injection of acetylcholine 1:5000 or stronger, subsequent doses at the optimum level are ineffective.[4]

The resistance of the response to atropine, the slight augmentation by eserine, and the paralyzing effect of high concentrations all pointed to the nicotine-like action of acetylcholine as being responsible for the pilomotor activity described. Choline, weak in nicotine-like action gives questionable responses. Mecholyl, until recently[5]

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[1] Kovacs, J., *Am. J. Med. Sci.*, 1934, **188**, 32.

[2] Hopkins, J. G., Kesten, B. M., and Hazel, O. G., *Arch. Dermatol. and Syphilol.*, 1938, **38**, 679.

[3] Battro, A., and Lanari, A., abstracted by *J. A. M. A.*, 1936, **107**, 890.

[4] Brücke, F. T., *Klin. Wochenschr.*, 1935, **14**, 7.

[5] Fellows, J., and Livingston, A. E., *J. Pharm. and Exp. Therap.*, 1939, **66**, 13.

thought to be devoid of any nicotine-like action, has a weak effect. Nicotine and alpha-lobeline both produce reactions identical with that by acetylcholine in optimum concentrations of $1:10^5$ and $1:10^6$ respectively.

The rapidly spreading and fleeting nature of the response suggested a mediation through a peripheral nervous mechanism. If the caudal nerves in a cat are cut the characteristic response may still be elicited. But if the nerves are allowed to degenerate local application of nicotine or acetylcholine fails to raise the hairs, though epinephrine is still effective since its action is directly on the muscles. If a piece of skin one centimeter square with its hair is excised from the dorsal surface of the tail and nicotine immediately injected a pilomotor reaction can be observed. This capacity disappears within 2 minutes after excision, though the response to epinephrine can be obtained after 15 minutes. Similarly, if 2 neighboring hair tufts are so close together that both erect when nicotine is injected under one of them, a cut just through the skin between them is sufficient to inhibit the erection of one when the injection is made under the other. If the lateral antibrachial cutaneous nerve in man is blocked by novocaine so that spontaneous physiological goose flesh does not appear in the area supplied by that nerve, nicotine and acetylcholine will still cause the typical pilomotion in the anesthetized area. It can, therefore, be concluded that the pilomotor phenomenon caused by these drugs depends on the integrity of an axon reflex pathway situated wholly within the skin.[6]

In the human skin subcutaneous infiltration of novocaine in dilutions up to 1:50,000, which produce no manifest reduction in cutaneous sensations, inhibits, in the area so treated, both the physiological goose flesh and that ordinarily caused by acetylcholine or nicotine. Mixtures of optimum concentrations of nicotine or acetylcholine with novocaine 1:200,000, apothesin 1:100,000, or cocaine 1:200,000 do not elicit any pilar erection.

It is evident that the nerve fibers carrying to the erector pili muscles impulses initiated by local application of acetylcholine and nicotine are adrenergic since: (1) a local anemia accompanies the goose flesh; (2) ergotamine inhibits the pilomotor response to acetylcholine and to lumbar sympathetic stimulation in the cat; (3) the behavior and appearance of the acetylcholine goose flesh is strikingly similar to that of the physiological phenomenon and to that of sympathetic stimulation, in which liberation of sympathin has been shown;[7] and (4) epinephrine in a concentration of $1:10^9$ injected intradermally in the cat's tail will produce a fleeting pilo-erection similar to that produced by nicotine, acetylcholine, lumbar sympathetic stimulation or physiological goose flesh stimuli.

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[6] A similar mechanism of a pilomotor response to faradic stimulation has been described by Lewis and Marvin, *J. Physiol.*, 1927, **64**, 87.

Summary. Experiments are cited which indicate that the local pilomotor action following intradermal acetylcholine occurs by virtue of its nicotine-like action, and that this drug as well as nicotine and other drugs possessing nicotine-like action exert this influence through an axon reflex the receptor end of which has several properties characteristic of autonomic ganglia and the effector end of which is evidently adrenergic.

10859 P

Nature of a Sweat Response to Drugs with Nicotine-like Action.

Julius M. Coon and Stephen Rothman. (Introduced by E. M. K. Geiling.)

From the Department of Pharmacology and Section of Dermatology, Department of Medicine, University of Chicago, Chicago, Illinois.

In a preliminary report on the nature of a local pilomotor response to acetylcholine injected intradermally[1] experiments have been cited which indicate that this pilomotor response occurs by virtue of the nicotine-like action of acetylcholine. The same effect can be obtained by nicotine and by alpha-lobeline. Drugs with nicotine-like effect act through an axon reflex in terminal branches of post-ganglionic sympathetic axons. The receptor end of the axon reflex behaves like an autonomic ganglion, whereas the motor end is evidently adrenergic.

An analogous phenomenon can be shown in the domain of cholinergic nerve endings. If 0.1 cc nicotine 1:100,000, alpha-lobeline 1:1,000,000, or acetylcholine 1:40,000 is injected intradermally in man, drops of sweat appear around the injection wheal in an area about 4 cm in diameter. The sweat secretion can be visualized easily by Minor's iodine-starch method.[2] At high room temperature and humidity and in persons sweating easily the sweat drops can be seen without an indicator.

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[7] Rosenblueth, A., and Cannon, W. B., *Am. J. Physiol.*, 1932, **99**, 398.

[1] Coon, J. M., and Rothman, S., Proc. Soc. Exp. Biol. and Med., 1939, **42**, 229.

[2] Minor, V., *Deutsche Z. f. Nervenheilk.*, 1927, **101**, 302.

The mechanism of this response differs from the direct action of acetylcholine on sweat glands. This is shown by the following facts: (1) intradermal acetylcholine in concentrations up to 10% causes local sweating whereas other drugs with nicotine-like action are effective only within a dilute range of concentration; (2) the sweat response to nicotine can be paralyzed by 1:200,000 dilutions of local anesthetics mixed with the nicotine, whereas the direct effect of acetylcholine is not impaired by them; (3) the local effect of acetylcholine originates in the injection wheal and spreads slowly into the neighborhood following the diffusion of the drug whereas the sweat secretion caused by nicotine appears as quickly in the outlying areas as on the wheal itself, corresponding to the effect of a nervous impulse.

Acetylcholine, then, apparently elicits sweat secretion in 2 different ways: (1) by direct effect on sweat glands and (2) by acting on nerve endings through its nicotine-like action. In high dilutions acetylcholine elicits sweating in the outlying areas, similarly to nicotine. This nicotine-like action of acetylcholine is proved by the fact that injections of acetylcholine in high concentrations inhibit the otherwise stimulating effect of a subsequent nicotine injection at the same point. This finding explains the observations on anhidrotic effects of high acetylcholine doses.[3, 4, 5] High nicotine concentrations also have a paralyzing effect.

Pilocarpine behaves similarly to acetylcholine in its direct action. If applied intradermally pilocarpine elicits sweating over the injection wheal, independent of concentration, not influenced by foregoing injection of highly concentrated nicotine and not influenced by local anesthetics.

In man, if 1 mg of atropine is administered the sweating response to nicotine is abolished or considerably weakened. The effect of atropine in this experiment corresponds to the paralyzing effect of ergotoxine on the pilomotor mechanism (1) whereas the direct effect of acetylcholine and pilocarpine on sweat glands corresponds to the direct effect of epinephrine on pilomotor muscles.

Blockade of the lateral cutaneous antibrachial nerve by procaine does not impair the sweat response to nicotine in the remote anesthetic area. However, in areas in which the corresponding nerve has degenerated, the sweat fails to appear after nicotine.

These findings furnish strong evidence for the axon reflex nature of the sweat response elicited. The receptor end of the axon reflex

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[3] Villaret, M., and Even, R., *Presse med.*, 1928, **2**, 1561.

[4] Ackermann, A., *Dermatologica*, 1939, **79**, 151.

[5] Hashimoto, extracted by (4), p. 156.

behaves like an autonomic ganglion whereas the effector end is cholinergic. From these experiments it cannot be determined whether receptor and effector ends are different anatomically and physiologically or all the ramifications of the postganglionic cholinergic sweat and adrenergic pilomotor fibers serve as receptors as well as effector ends.

The question arises as to whether this axon reflex mechanism plays a rôle in physiological responses to external cutaneous pilomotor and sweat stimuli. The primary confinement of these cutaneous responses to the area surrounding the point of stimulation, and their secondary unilateral and bilateral expansion seem to indicate that primary axon reflexes are followed by long spinal reflexes.

Summary. Localized sweat secretion can be elicited by intradermal injection of drugs with nicotine-like action. This effect is accomplished through a peripheral axon reflex mechanism which is easily differentiated experimentally from the direct muscarine action of acetylcholine on the sweat glands.

10860

Reduction of Dehydroascorbic Acid in the Stomach and Small Intestine.*

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From the Department of Chemistry, University of Pittsburgh, Pa.

In view of the fact that a portion of the Vitamin C intake of animals and man may be in the form of dehydroascorbic acid,[1-4] it is of interest to know whether the unstable reversibly oxidized form can be utilized efficiently, and whether there is a significant degree of reduction to ascorbic acid in the intestinal tract. It is clearly recognized that dehydroascorbic acid is effective as an antiscorbutic agent,[5-8] but there has been wide variation in the results from different laboratories regarding (a) the protective dosage required and (b) the relative utilization when administered by ingestion and injection.

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* Contribution No. 390 from the Department of Chemistry, University of Pittsburgh. The authors are indebted to the Buhl Foundation for a research grant that has made the present investigation possible.

[1] Bessey, O. A., *J. Biol. Chem.*, 1938, **126**, 771.

[2] Reedman, E. J., and McHenry, E. W., *Biochem. J.*, 1938, **32**, 85.

[3] Tressler, D. K., *et al.*, *Food Research*, 1938, **3**, 133, 311, 403, 409.

[4] Fujita, A., and Ebihara, T., *Biochem. Z.*, 1937, **290**, 201; Fujita, A., and Sakamoto, T., *ibid.*, 1938, **297**, 10.

The tendency toward irreversible decomposition of dehydroascorbic acid[9, 10] is such that utilization from the intestinal tract would be greatly influenced by (a) the low pH range of the stomach contents, (b) a rapid reduction to ascorbic acid by the contents of the intestinal tract, and (c) the rate of assimilation from the intestinal tract, followed by, or coincident with, reduction to the stable form. Biological assays carried out in our own laboratory[11] indicate that dehydroascorbic acid is nearly as effective antiscorbutically as ascorbic acid when administered with sufficient care, either orally or parenterally. The time factor in manipulating the reagents, pH of reagents, and the condition of the animals no doubt all have an important bearing upon the results of such assays.

In a previous communication[12] the reduction of dehydroascorbic acid by guinea pig tissues was studied in detail, with the conclusion that diffusible and fixed sulfhydryl compounds were responsible for nearly all of the reducing action, without the interposition of enzymic activity. The products of protein digestion have also been shown to exert a protective action toward the reduced form of the vitamin.[13]

From the data in Table I it is evident that the contents of the small intestine may bring about a rapid reduction of small amounts of dehydroascorbic acid at pH 7.2. The effective blocking action of iodoacetate indicated again that sulfhydryl groups were responsible for most of the reducing action.

The guinea pigs used were 600 to 800 g in weight, maintained on the standard Sherman diet (rolled oats, bran, butter fat, and heated milk powder), supplemented with spinach until 3 to 4 days before their use for reduction tests. The contents of the stomach and small intestine were removed rapidly at the designated time and made up to standard volume with 0.1 M McIlvaine buffer. Aliquot samples were placed in Thunberg tubes, evacuated, and allowed to stand

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[5] Tillmanns, J., Hirsch, P., and Siebert, F., *Z. Untersuch. Lebensm.*, 1932, **63**, 21.

[6] Demole, I., *Z. physiol. Chem.*, 1933, **217**, 83.

[7] Hirst, E. L., and Zilva, S. S., *Biochem. J.*, 1933, **27**, 1271.

[8] Fox, F. S., and Levy, L. F., *Biochem. J.*, 1936, **30**, 211.

[9] Borsook, H., Davenport, H. W., Jeffries, C. E. P., and Warner, R. C., *J. Biol. Chem.*, 1937, **117**, 237.

[10] Ball, E., *J. Biol. Chem.*, 1937, **118**, 219.

[11] From the unpublished work of S. C. Camp and T. M. Gorski.

[12] Schu'tze, M. O., Stotz, E., and King, C. G., *J. Biol. Chem.*, 1938, **122**, 395.

[13] Stotz, E., Harrer, C. J., and King, C. G., *J. Biol. Chem.*, 1937, **119**, 511.

TABLE I.

Reduction of Dehydroascorbic Acid by One-third of Contents of the Small Intestine.

Anaerobic, 37.5°, pH 7.2, 2 mg of dehydroascorbic acid added, values expressed in each case as mg of ascorbic acid.

Time min	Titration equivalent of intestinal contents alone mg	Titration value in the presence of iodoacetate and dehydroascorbic acid mg	Intestinal contents and added dehydroascorbic acid mg	Reduction by contents of intestine %
5-6	.089	.101	.164	3.8
	.081	.127	.254	8.7
	.116	.138	.278	8.1
	.155	.129	.308	7.2
	.193	.139	.303	5.5
Avg	.127	.127	.261	6.7
8-10	.089	.101	.228	7.0
	.081	.289	.289	10.4
	.116	.138	.312	9.8
	.112	.125	.363	12.5
	.155	.179	.385	11.5
	.193	.167	.414	11.1
	.075	.083	.343	13.4
Avg	.117	.155	.333	10.8
30	.089	.101	.317	11.4
	.090	.156	.400	15.5
	.086	.128	.285	9.9
	.060	—	.339	13.9
	.124	—	.332	10.4
	.161	—	.444	14.2
	.155	.179	.434	13.9
	.193	.167	.524	16.5
Avg	.119	.143	.384	13.2

at 37.5° for 30 min., with and without added iodoacetate. Iodine-oxidized dehydroascorbic acid (2 mg) was added from the overhang, the total volume being 11 cc. Control tests were run with dehydroascorbic acid alone, and with the intestinal contents alone, and the corresponding corrections made for the data in Table I. The titrations were made with 2,6-dichlorophenolindophenol, using 2% metaphosphoric and 8% trichloracetic acid, as outlined in earlier publications.[14]

Stomach contents at their natural pH, approximately 2.2, did not exert an appreciable reduction (<0.5% in 30 min.). When the pH of the stomach contents was raised to 7.2, however, the reducing action was approximately one-third that of the contents of the small intestine, on a comparable total solids basis.

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[14] Bessey, O. A., and King, C. G., *J. Biol. Chem.*, 1933, **103**, 687; Musulin, R. R., and King, C. G., *J. Biol. Chem.*, 1936, **116**, 409.

In biological tests involving analyses of stomach and intestinal contents at frequent intervals after feeding dehydroascorbic acid, there was no evidence of a marked change in the stomach—neither reduction, decomposition, nor absorption was evident. In the small intestine, studied in one-third length segments, absorption and reduction were too rapid and irregular to follow satisfactorily with consistent quantitative data. The results with the stomach and intestine were in agreement, however, with the *in vitro* studies of reduction and stability, and also with the assay findings regarding antiscorbutic activity.

10861 P

Preferential Mottling of the Enamel of Rat Molars.

SARA F. DIXON AND GERALD J. COX. (Introduced by C. G. King.)

From the Nutrition Fellowship of The Buhl Foundation, Mellon Institute, Pittsburgh, Pa.

Cox, Matuschak, Dixon, and Walker[1] produced mottled enamel in rat molars by daily individual feedings from birth of relatively high doses of fluorine to suckling rats. The first two molars, which normally are extruded at 19 days, were affected; the third molars, which appear on the thirty-fifth day, were apparently normal.

Miller[2] has shown that the development of dental caries is inhibited by 250 p.p.m. of sodium fluoride or 500 p.p.m. of calcium fluoride in a caries-producing ration fed to rats 28 days old. His ration was based on coarse rice.

In a confirmatory study of Miller's finding a ration of corn meal 66, whole milk powder 30, alfalfa meal 3, and sodium chloride 1 was fed to rats weaned at 21 days. Two groups, 22 and 23 rats, respectively, made up of litter mates, were used. One group had 250 p.p.m. of sodium fluoride in the ration. All were sacrificed after 8 weeks.

On examination of the teeth before they were sectioned, it was found that the enamel of the third molars of 19 out of 22 rats that had received added fluorine showed a faint diffuse milkiness. None of the first or second molars was affected. No milkiness was observed in any teeth of the control rats. No difference could be seen in the enamel when the teeth were sectioned by the method of Cox and Dixon.[3]

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[1] Cox, G. J., Matuschak, M. C., Dixon, S. F., and Walker, W. E., *Science*, 1939, **90**, 83.

[2] Miller, B. F., PROC. SOC. EXP. BIOL. AND MED., 1938, **39**, 389.

This discovery of mottled enamel in the third molars of rats makes it possible to produce preferential decay in a rat mouth if the protective action of fluorine[4] is exerted by such late inclusion in the diet. On the other hand, failure to prevent caries in the third molar would indicate that fluorine exerts its protective action earlier in tooth life. Similarly, other substances, for example, Vitamin D, can be used in a study of their relation to dental caries.

10862

Circulation Time Under Conditions of Work and Rest in Subjects with Normal and Abnormal Hearts.*

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The work of Blumgart, Weiss, and their associates on circulatory dynamics gave impetus to the investigation of the circulation time.[1] Since publication of their reports, it has been shown that the circulation time is influenced by exercise, the basal metabolism, digestion, the viscosity of the blood, the number of red blood corpuscles, the condition of the respiratory organs, the functional state of the myocardium, and many vasomotor factors.[2] Lucia and Aggeler[3] have shown that the intramuscular injection of adrenalin markedly shortens the circulation time. These factors cause fluctuations sufficient to cast doubt on the value of a single observation or uncontrolled measurements of the circulation time.

The substances in most common use for the subjective determination of the circulation time are calcium gluconate, decholin, saccharin, and ether; those for the objective determination include radioactive

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[3] Cox, G. J., and Dixon, S. F., *J. Dent. Res.*, 1939, **18**, 153.

[4] Cox, G. J., Matuschak, M. C., Dixon, S. F., Dodds, M. L., and Walker, W. E., in press.

* Assisted by a grant from the Christine Breon Fund.

[1] Blumgart, H. L., *et al.*, *J. Clin. Invest.*, 1927, **4**, 1, 15, 149, 173, 199, 389, 555.

[2] Kunkel, P., Stead, E. A., and Weiss, S., *J. Clin. Invest.*, 1939, **18**, 225.

[3] Lucia, S. P., and Aggeler, P. M., unpublished data.

substances and fluorescein. Perhaps alpha-lobelin, sodium cyanide and paraldehyde belong to this latter group.

The values usually accepted for the circulation time of normal and abnormal subjects fluctuate through wide ranges.[4] The variation in circulation time for a given disease condition is as great as the variations between different individuals. Master's work[5] on exercise tolerance suggested to us that perhaps the changes in circulation time induced by exercise might give some clue to the state of the myocardial reserve. Accordingly, we devised a method whereby a subject is given a measured amount of exercise, and the circulation time is obtained before and after the exercise. This study was carried out in normal and pathologic subjects before and immediately after exercise, and also before and after a given period of rest.

Method. With the patient in the supine position, 5 cc of 20% calcium gluconate† were injected rapidly through an 18-gauge needle into an antecubital vein at the level of the midaxilla. The time elapsing between the end of injection and the subjective sensation of warmth at the base of the tongue (the circulation time) was meas-

TABLE I.
Circulation Time of Normal Subjects Before and After Exercise.

Case No.	Circulation Time (in sec) before	Circulation Time (in sec) 5 min after	Difference (in sec)
1	13.0	11.6	—1.4
2	16.8	13.8	—3.0
3	15.2	10.1	—5.1
4	12.6	10.4	—2.2
5	14.4	12.4	—2.0
6	13.0	9.5	—3.5
7	15.0	12.4	—2.6
8	17.0	15.4	—1.6
9	15.0	13.0	—2.0
10	15.2	14.2	—1.0
11	13.7	13.9	+0.2
12	11.7	13.0	+1.3
13	13.2	11.1	—2.1
14	10.6	9.9	—0.7
15	11.1	8.3	—2.8
16	16.4	14.5	—1.9
17	10.0	9.0	—1.0
18	14.8	9.8	—5.0
19	12.2	10.0	—2.2
20	15.6	9.6	—6.0
Mean	13.8	11.6	—2.03

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[4] Baer, S., and Slipakoff, B. G., *Am. Heart J.*, 1938, **16**, 29.

[5] Master, A. M., *Am. Heart J.*, 1935, **10**, 495.

† We wish to acknowledge with thanks the cooperation of the Sandoz Chemical Company in furnishing to us the Neo-Calglucon (brand of calcium gluconate) used in these experiments.

TABLE II.
Circulation Time of Abnormal Subjects Before and After Exercise.

Case No.	Circulation Time (in sec) before	Circulation Time (in sec) 5 min after	Difference (in sec)	Diagnosis
1	13.9	12.4	—1.5	Hypertension
2	15.9	13.2	—2.7	”
3	17.4	15.5	-1.9	Auricular Fibrillation, Hypertension
4	14.6	11.3	—3.3	Hypertension
5	13.8	10.8	—3.0	Artscl. Heart Disease
6	8.1	7.0	—1.1	? Hyperthyroid
7	19.0	16.0	—3.0	Artscl. Heart Disease
8	19.1	15.1	—4.0	Luetic Heart Dis., Aortic Insuf.
9	10.3	9.5	—0.8	Hypertension
10	18.3	11.1	—7.2	Bundle Branch Block
11	20.1	16.4	—3.7	Artscl. Heart Disease
12	23.0	18.7	4.3	Hypertension
13	18.0	13.3	—4.7	”
14	25.9	16.3	-9.3	Auric. Fibrillation, Hypertension
15	20.3	13.4	-6.9	Luetic Heart Disease
16	13.6	11.5	--2.1	Hypertension
17	23.0	19.4	—3.6	Hypertensive
18	28.9	22.9	—6.0	Artscl. Heart Disease
19	12.5	9.8	2.7	Rheumatic Heart Disease
20	18.4	13.3	-5.1	Artscl. Heart Disease
21	16.1	14.3	1.8	” ” ”
22	17.3	15.3	--2.0	” ” ”
23	13.7	9.9	-3.8	Hypertension
24	31.4	17.0	14.4	” Auric. Fibrillation
25	19.5	17.4	2.1	Undiagnosed
26	14.6	10.6	-4.0	Luetic Heart Disease
27	14.6	16.5	+1.9	Artscl. ” ”
28	19.8	19.0	--0.8	Rh. Ht. Dis., Mit. Sten., Aur. Fib.
29	14.5	14.5	—0.0	Hypertension
30	11.1	11.5	+0.4	Undiagnosed
31	22.1	22.0	—0.1	Luetic Ht. Dis., Aortic Insuf.
32	14.6	14.5	—0.1	Undiagnosed
33	19.0	19.0	0.0	Hypertension
34	22.8	23.5	+0.7	Luetic Ht. Dis., Aortic Insuf.
35	17.3	21.4	+4.1	Artscl. Heart Disease
36	14.6	15.2	+0.6	Undiagnosed
37	14.6	15.8	+1.2	”
38	14.6	14.5	—0.1	Artscl. Heart Disease
39	12.6	10.0	2.6	Hypertension
40	14.8	12.4	—2.4	Luetic Heart Dis., Aneurysm
41	15.2	13.8	·1.4	Artscl. Heart Disease
42	17.8	16.0	1.8	Hyperten. Ht. Dis., Auric. Fib.
43	20.0	18.6	—1.4	Artscl. Heart Disease
44	14.8	13.2	1.6	” ” ”
45	17.0	26.8	+9.8	” ” ” , Angina
46	14.4	11.4	—3.0	” and Hyperten. Ht. Dis.
47	13.0	11.0	—2.0	” Heart Dis., Angina
48	14.8	12.2	—2.6	? Coronary Occlusion
Mean	16.9	14.6	-1.9	

ured with a stop watch. Subsequent determinations were made following injection at the site of the original venipuncture.

Those patients who were subjected to work were directed to make

25 round trips over a two-step lift[5] in a period of 5 minutes, the speed being controlled by a metronome. With few exceptions, all subjects had the same amount of exercise. Patients in the control groups were maintained at rest in the initial position for 5 minutes.

Data: Normal subjects before and after exercise. (Table I.) After exercise, the circulation time was shortened, the mean value being 2.03 seconds, indicating a more rapid circulation. In terms of the fluctuations within the group, the mean value is apparently insignificant.

Abnormal subjects before and after exercise. (Table II.) The subjects were a heterogeneous group of patients suffering from various illnesses associated with slight to severe alteration of cardiac function. After exercise, the circulation time was shortened, the mean value being 1.9 seconds, indicating a more rapid circulation. In terms of the fluctuations within the group, this value is also insignificant. Inspection of Table II reveals that the circulation time is not characteristic of the type of disease, with the possible exception of auricular fibrillation. This fact, we believe, may account in part for the wide fluctuations within the group. This conclusion is further borne out by the data of Hamilton and Cannon,[6] who used radio-

TABLE III.
Circulation Time of Normal Subjects Before and After Rest.

Case No.	Circulation Time (in sec)		Difference (in sec)
	before	5 min after	
1	14.4	13.0	—1.4
2	12.2	10.8	—1.4
3	11.4	11.2	—0.2
4	11.4	10.4	—1.0
5	9.0	7.4	—1.6
6	12.6	10.2	—2.4
7	11.2	12.3	+1.1
8	12.0	11.8	—0.2
9	8.5	9.6	+1.1
10	14.8	14.2	—0.6
11	12.4	12.0	—0.4
12	14.8	14.0	—0.8
13	11.8	11.0	—0.8
14	11.2	8.0	—3.2
15	10.6	8.2	—2.4
16	13.0	15.6	+2.6
17	11.4	16.4	+5.0
18	9.4	12.0	+2.6
19	9.6	10.6	+1.0
20	12.5	10.0	—2.5
21	7.0	6.6	—0.4
Mean	11.6	11.2	—0.28

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[6] Hamilton, J. G., and Cannon, E. F., unpublished data.

TABLE IV.
Circulation Time of Abnormal Subjects Before and After Rest.

Case No.	Circulation Time (in sec) before	Circulation Time (in sec) 5 min after	Difference (in sec)	Diagnosis
1	10.2	11.2	+ 1.0	Rh. Ht. Dis., Aortic Insuf.
2	51.3	56.8	+ 5.5	Auric. Fib. ? Etiology
3	14.4	14.6	+ 0.2	Rh. Ht. Dis., Aortic Insuf.
4	11.2	11.0	— 0.2	Artscl. Heart Disease
5	42.0	49.8	+ 7.8	Coronary occlusion
6	12.2	13.0	+ 0.8	Artscl. Heart Disease
7	8.6	7.8	— 0.8	Hyptertension
8	53.2	52.2	— 1.0	Artscl., Hyperten. Ht. Dis.
9	30.6	28.0	— 2.6	Artscl. Ht. Dis., c̄ mild fail.
10	9.6	8.6	— 1.0	Aortic coarctation
11	19.6	19.4	— 0.2	Artscl., Hyperten. Ht. Dis.
12	11.4	11.4	— 0.0	” ” ” ”
13	10.5	9.6	— 0.9	” ” ” ”
14	10.2	10.4	+ 0.2	” ” ” ”
15	11.2	11.0	— 0.2	Ht. Disease, ? etiology
16	12.2	15.0	+ 2.8	Artscl. Hyperten. Ht. Dis., Auri. Fib.
17	17.8	16.6	— 1.2	Paroxysmal Aur. Fib.
18	54.0	39.6	—14.4	Artsl. Ht. Dis., Aur. Fib.
19	17.2	13.4	— 3.8	Ht. Dis., ? etiology
20	8.4	8.6	+ 0.2	Artscl. Heart Disease

sodium and decholin for a comparison of the circulation times of these substances in the same individual. A comparison of the mean values of Groups I and II reveals no significant variations, with the exception that on the average the circulation time of patients with heart disease was prolonged. Insofar as these subjects are concerned, no information of value was obtained by means of this test.

Normal subjects before and after rest. (Table III.) The circulation time for this group remained practically unchanged, the mean difference between tests performed before and after a 5-minute rest period was 0.28 seconds.

Abnormal subjects before and after rest. (Table IV.) In this group there were marked fluctuations between individual values; however, the mean difference between tests performed before and after a 5-minute rest period was only 0.38 seconds. On the average the circulation time was relatively prolonged in these subjects with

TABLE V.
Summary of Mean Circulation Time in Normal and Abnormal Subjects Before and After a 5-Minute Period of Work or Rest.

	No. of Subjects	Circulation Time (in sec) before	Circulation Time (in sec) 5 min after	Difference (in sec)
Normal subjects with work	20	13.8	11.6	2.03
Abnormal ” ” ”	48	16.9	14.6	1.90
Normal subjects at rest	21	11.6	11.2	0.28
Abnormal ” ” ”	20	20.8	20.0	0.38

heart disease, when contrasted with normal subjects. A summary of the significant data is given in Table V.

Summary. The circulation time is characteristic for the subject rather than for the type of heart disease. On the average, the circulation time for individuals with heart disease is prolonged. The difference between the circulation time before and after 5 minutes of exercise is greater than that before and after a 5-minute period of rest. The difference between the circulation time before and after 5 minutes of exercise in the diseased group is similar to that of the normal group. The difference induced in the circulation time after exercise is not significant as a test of cardiac efficiency.

10863

Comparison of Different Types of Central Stimulation from Analeptics.*

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In a study of the analeptic potency of the sympathomimetic amines,[1] a simple and rapid method has been developed in this laboratory for graphic registration of one kind of central stimulation. In order to establish standards of comparison for this study, observations have been made on 5 unrelated analeptics, to determine whether the method will demonstrate differences between the various compounds, which could serve as a basis for analysis of their actions. The present report gives the results on the stimulant actions of metrazol, picrotoxin, coramine, caffeine, and cocaine, as revealed by this procedure. Later, results of the application of this same method to the sympathomimetic amines will be reported.

After testing a considerable variety of methods for measuring the total activity of animals, one was devised which utilizes well known principles, although somewhat differently applied. It has the advantage of graphically integrating total activity, without the necessity of laboriously measuring marks of a lever on a drum, as is done

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* Supported, in part, by Grant 345 of the Committee on Therapeutic Research of the Council on Pharmacy and Chemistry of the American Medical Association.

[1] Tainter, M. L., Whitsell, L. J., and Dille, J. M., *J. Pharm. Exp. Therap.*, 1939, **67**, 56.

with the usual types of apparatus.[9] A white rat is placed in a small wire cage 6 inches square, which is suspended from a spring, and moves up and down in response to each movement, or shift in balance, of the animal. A suitable spring for this purpose is like that used in window blinds, since it is made of well tempered steel, does not change its elasticity, and has the proper degree of sensitivity for appropriate records. This movement is summated and recorded by connecting the spring to a Harvard work adder. With each revolution of the work adder, electrical contact is made through a mercury cup, recording on a kymograph through a signal magnet in the usual way. Twenty such units are used, with the cages suspended in a constant temperature chamber of 27 to 29°C, so the animals may be kept warm and at nearly basal conditions. The chamber is brightly lighted, since the rats are more quiet in the light than in darkness, their normal period of activity. Groups of 10 rats are used in each series, one-half being injected subcutaneously with normal saline solution (0.85% NaCl), and the other half with a test solution, and their activity recorded continuously until it returns to normal. One week later, the same rats are reinjected, with the groups reversed to give a "cross-over" test. Care is taken that each animal is put in the same cage, so that any differences in sensitivity of the individual recording units are balanced out by the method of controls used. This latter precaution does not appear to be particularly important since the apparatus is very uniform.

From the total values of the recorded movements observed in each half hour after the amines are injected there are subtracted the amounts of activity shown by the same animals after the salt solution injections. The net revolutions by the 10 rats are averaged to give the general curve of response to the dose in question. The dosages of the drugs used started from those which were completely ineffective, and increased by increments of 100% until ones were reached which caused convulsions or death. Since this is a severer reaction than is desirable for ordinary therapeutic use, it was not thought worthwhile, for the present study, to test larger ones.

The results are summarized graphically in Fig. 1.

It is clear that metrazol and picrotoxin behaved quite differently from coramine and caffeine. These former agents produced no significant increases in activity over the control levels, except when, with 40 mg of metrazol per kilo, 7 of the 10 rats had convulsions, and with 1.6 mg picrotoxin, 5 of the 10 rats showed the same response. However, no animals died in these 2 series. The convulsions resembled those commonly ascribed to spinal and medullary stimulation. They lasted about 15 seconds for each seizure and recurred for 30 minutes. Thereafter the animals showed no more activity than the control spontaneous variations. It appears, therefore, that metrazol and picrotoxin do not increase the total activity of rats, as measured by this type of apparatus, unless convulsant or fatal doses are used.

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[9] Shirley, M., *Psychol. Bull.*, 1929, **26**, 341.

On the other hand, coramine, caffeine, and cocaine presented quite a different picture. Twenty mg of coramine per kilo increased the activity only slightly above that of the control animals, with 40 mg there was a definite stimulation, and with 160 mg there was an increase up to 12 revolutions per hour, for about 7 hours. This latter dose caused convulsions in 2 rats. Similarly, caffeine showed what is probably a threshold response to 10 mg per kilo, and a very marked response of 22 revolutions per hour to 40 mg per kilo, with one fatality. Experiments with cocaine (not shown in Fig. 1) were carried out on 6 animals for each dose with similar results. Doses of 50 and 60 mg per kilo produced an increase of about 40 revolutions per half hour without the appearance of convulsions. Maximum activity was reached at the end of one hour, and activity returned to normal at the end of about 3.5 hours. One of the six rats died from 60 mg of cocaine.

The animals receiving coramine, caffeine and cocaine in effective doses showed a rather characteristic behavior. They moved constantly around the cage, rubbed their noses, scratched and gnawed at the meshed wire, in a continuous coördinated muscular activity of a type resembling that of a purposeful or psychic "drive". This was strikingly different from that of the convulsions of picrotoxin and metrazol, and was probably initiated from levels higher than the medulla.

A large body of published observations agree that caffeine in appropriate doses, can shorten reaction time, increase the speed of discrimination and other similar criteria of cortical activity.[2, 3, 4, 10] In addition, Miller and Miles[5] have shown that rats, injected with 50 mg per kilo of caffeine sodium benzoate, ran faster in response to the psychic drive of hunger. This dose of caffeine sodium benzoate is of the same order of magnitude as the doses used by us, which also resulted in a marked increase in coördinated activity. Similar effects have been described by others for coramine, and are well summarized

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[2] Eddy, N. V., and Downs, A. W., *J. Pharm. Exp. Therap.*, 1928, **33**, 167.

[3] Cheney, R. H., *J. Pharm. Exp. Therap.*, 1935, **53**, 304.

[4] Horst, K., and Jenkins, W. L., *J. Pharm. Exp. Therap.*, 1935, **53**, 385.

[10] Skinner, B. F., and Heron, W. T., *Psychol. Record*, 1937, **1**, 340.

[5] Miller, N. E., and Miles, W. R., *J. Comp. Psychol.*, 1935, **20**, 397.

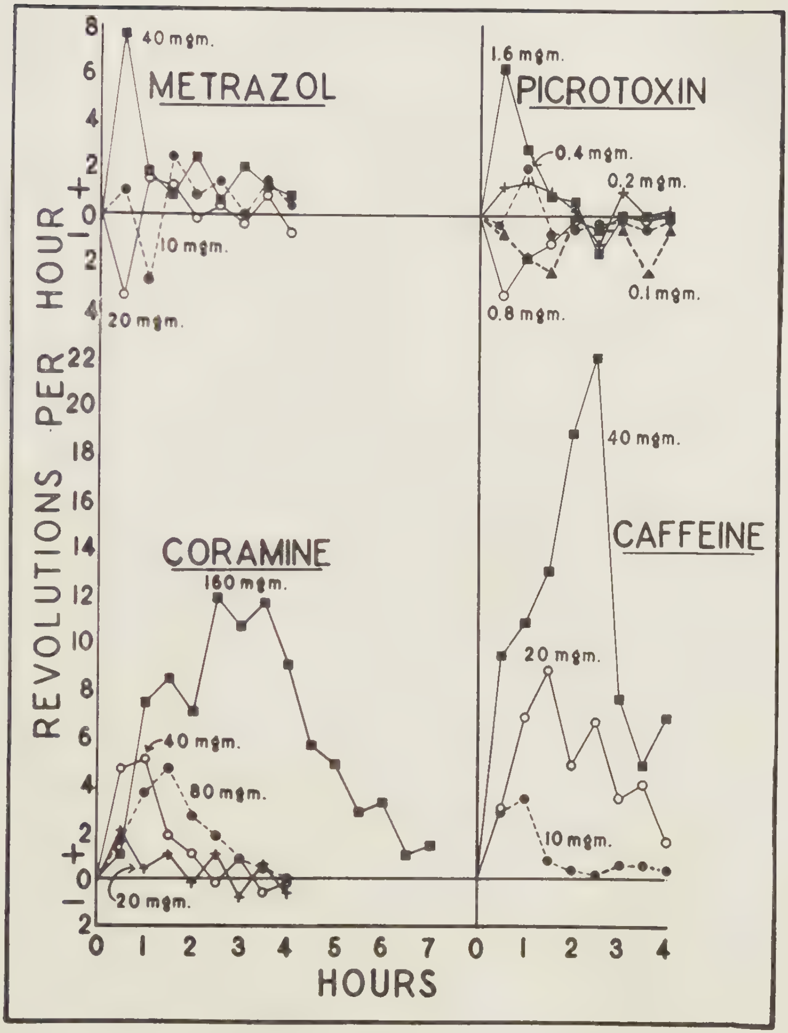

FIG. 1.

Stimulation of spontaneous muscular activity through the central nervous system in white rats produced by metrazol, picrotoxin, coramine and caffeine sodiobenzoate.

Each curve represents average net activity of 10 rats injected subcutaneously with the dose of the analeptic indicated, expressed in mg per kilogram.

by Hildebrandt.[6] For instance, according to Uhlmann, rabbits given this drug become excited, jump around, and show spontaneous activity of various types. Apparently, all parts of the brain, as well as the spinal cord, are almost equally sensitive to it. This phase of cerebral stimulation has not been generally emphasized, inasmuch as the majority of workers have been interested primarily in the effects of these analeptics on respiration, circulation, or the recovery of consciousness. While the actions of picrotoxin are predominantly localized in the medulla, the stimulant action can extend below this area. Our results do not reveal supramedullary stimulation by subconvulsant doses, at least not of the kind which gives rise to spontaneous and coördinated muscular movements.

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[6] Hildebrandt, F., *Heffter's Handbuch der Experimentellen Pharmakologie,* 1937, **5**, 128.

Metrazol was investigated by Jackson[7] from this viewpoint with the general result that the stimulants were not equally effective against various depressants, and metrazol caused tremors and convulsions with very little specific stimulation of the cerebral cortex. According to Hildebrandt's summary[8] its main effect consists in excitation of the central nervous system, with convulsions after large doses. However, it is not clear whether the drug affects the cerebral cortex or the psychic areas, rather than more caudal zones. Hildebrandt quotes Schoen, who attempted to localize the seat of action of metrazol by removing the cerebrum above the thalamus, but did not produce any alteration in metrazol-activity. This indicated that a considerable part of the metrazol stimulation was not derived from the higher levels of the nervous system. Winniwarter is quoted by Hildebrandt to the effect that typical convulsions are produced by metrazol in the decerebrate rabbit, thus indicating that metrazol attacks mainly the sub-cortical centers. Our results with metrazol are in agreement with those of previous workers, inasmuch as we failed to demonstrate any cortical type of stimulation, according to the criteria used.

Taking our results as a whole, it appears that metrazol and picrotoxin in doses below the convulsive level, do not produce increased motor activity, whereas coramine, caffeine, and cocaine cause increased activity of a coördinated voluntary purposeful type, and of an intensity proportionate to the dosage. Generalized convulsions are produced by those latter drugs only as the expression of an overwhelming degree of stimulation. Accordingly, the method used by us permits the differentiation of these two types of central stimulation in a rather clear manner, and is believed to be applicable in studies of other central stimulants. The method is also applicable to drug addiction studies as a means of obtaining record of changes in activity during habituation and withdrawal.

Summary and Conclusions. 1. A method is described for measuring stimulation produced by analeptics, which consists of suspending white rats or other small animals in small cages from wire springs and summating the amount of movement by connecting the springs to Harvard work adders. The activities resulting over periods of hours, are recorded by electrical contacts on the work adders through signal magnets writing on a kymograph drum. The apparatus is kept in a lighted constant temperature chamber between 27 and 29°C as conditions for minimum activity. The amounts of activity shown by the animals after injections of normal saline solution are designated as control levels of activity, and subtracted from those observed during the action of a drug to give the net stimulation produced by the agent in question.

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[7] Jackson, D. E., *J. Lab. Clin. Med.*, 1934, **20**, 1.

[8] Hildebrandt, F., *Heffter's Handbuch der Experimentellen Pharmakologie*, 1937, **5**, 151.

2. Metrazol, in doses from 10 to 40 mg per kilo, produced no increase in general activity except during convulsions resulting from doses of 40 mg. Picrotoxin, in doses from 0.1 to 1.6 mg per kilo also produced no increase in activity, except after doses of 1.6 mg, when the animals were in generalized convulsions. Therefore, these two agents showed no evidence of stimulating the higher levels of the nervous system, particularly the areas for purposeful coördinated movement.

3. Coramine, in doses of 20 mg per kilo, produced just perceptible degrees of increase of coördinated and purposeful activity, and with 40, 80, and 160 mg doses these changes were so large as to be unmistakable. The highest dose caused generalized convulsions in some animals. The actions of 160 mg persisted for about 7 hours, whereas those of 40 and 80 mg lasted 2½ and 4 hours, respectively.

4. Caffeine sodiobenzoate in doses of 10 mg per kilo, produced threshold increases in activity similar to that of coramine. Twenty mg per kilo caused larger increases and 40 mg produced very marked stimulation. The highest dose killed one of the 10 animals, so that higher dosage levels were not tested.

5. Cocaine in doses of 50 and 60 mg per kilo caused very marked stimulation resembling that of the highest doses of coramine and caffeine. The larger dose resulted in the death of one of the six animals used for this drug.

6. The margin of safety between the barely effective and convulsive or fatal doses of coramine was 8 times, and for caffeine, 4 times, indicating a narrower range for the latter drug under these conditions.

7. Therefore coramine, caffeine and cocaine stimulate activity in rats in a manner distinctive from that of picrotoxin and metrazol. The method differentiates between two kinds of central nervous system stimulation, and is suitable for study of the analeptic potency of

other agents, and for studies requiring measurement of spontaneous activity, such as in drug addiction.

10864

A Gas Machine for the Anesthetization of Small Laboratory Animals.*

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In connection with a problem dealing with the effects of various anesthetic gases on small laboratory animals (rabbits), it was necessary in the interest of safety and economy to construct an apparatus which could be attached to any standard clinical gas machine. After considerable difficulty, an apparatus meeting these requirements was designed. This apparatus makes it possible to supply anesthetic gases to the animal in rather constant concentrations.

The apparatus (Fig. 1) consists of a small soda-lime cannister and 2 flutter valves, mounted on a base 8 by 6 inches. Accurate quantities of the gases are measured by a large gas machine and are delivered into the small machine through the inlet tubing (A). The gases then pass over the flutter valve (B) and are delivered into the face mask. The exhaled gases pass through the flutter valve (F) and then through a small soda-lime cannister into the rebreathing bag. On inspiration, the mixture of gases from the rebreathing bag passes through the inspiratory flutter valve (B) to the animal. The small chamber above this flutter valve acts as a mixing chamber for the fresh and the rebreathed gases.

This constitutes a closed carbon dioxide absorption circuit and allows rebreathing of the anesthetic gases. The flutter valves (B and F) alternately open and close on inhalation and exhalation, thus compelling all gases to pass in one direction only. The rebreathing bag is a small rubber bag of 400 cc capacity. The metal bell-shaped face mask is covered with a rubber diaphragm in the center of which is a small opening to admit the nose of the animal. The tubing has an inside diameter of 8 mm. This apparatus permits small animals to be anesthetized with a minimal amount of anesthetic gases. The resistance of the gases passing through the machine is less than 2 cm

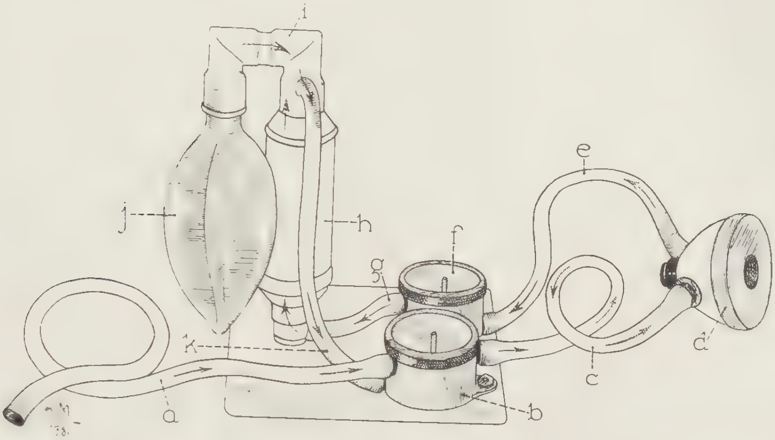

FIG. 1.

Gas machine for anesthetizing small laboratory animals.

(A) Intake tubing
(B) Intake flutter valve.
(D) Face mask.
(F) Exhaling flutter valve.
(H) Soda-lime cannister.
(J) Rebreathing bag.
(K) Inlet for gases from rebreathing bag.
(The arrows mark the course of the gases in the rebreathing circuit.)

water pressure, thus allowing the animal to breathe with a minimum of exertion. The use of the apparatus in experiments on rabbits proved highly satisfactory and with slight modification of the face mask, the apparatus can be used on any other small laboratory animal. We believe a similar machine can be satisfactorily adapted to the anesthetization of very small children.

10865

Blood Pressures in Experimental Hydronephrosis.

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The studies of numerous investigators[1, 2, 3] have suggested that reduction in the amount of functioning renal tissue in dogs is ac-

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[1] Passler, H., and Heineke, D., *Verhandl. d. deutsch. path. Gesell.*, 1905, **9**, 99.

[2] Mark, R. E., *Z. f. Exp. Med.*, 1925, **46**, 1; 1928, **59**, 601.

[3] Friedman, L., and Wachsmith, W., *Arch. f. Exp. Path.*, 1930, **101**, 173.

companied by a moderate rise in blood pressure. A similar rise has also been obtained by permanent obstruction of the ureter.[4, 5] The present study indicates, however, that when the reduction in functioning renal tissue is accomplished by means of experimental chronic hydronephrosis, produced by the partial constriction of the ureter, no rise in blood pressure occurs.

The experimental hydronephrosis in dogs was produced in one kidney by the method previously described,[6] followed by removal of the opposite kidney. The animals were roughly divided into 2 groups on the basis of physical symptoms, pyelographic findings, gross and microscopic pathology of the hydronephrotic kidneys, and blood findings: (1) The *chronic* group included animals in which intravenous pyelograms showed the disappearance of the calices of the kidney without further progression over a period of months, while no physical symptoms developed, the blood serum showed no significant deviation from normal, and a considerable amount of functioning renal tissue remained; these animals were in a non-uremic stage of hydronephrosis. (2) The *progressive* group included animals which developed signs and symptoms of severe renal involvement; the greater part of the renal tissue was destroyed—therefore excretory urographic shadows could not be obtained—and the animals progressed into the uremic stage of hydronephrosis, with marked acidosis and retention of non-protein nitrogen and inorganic phosphorus.

The mean blood pressures of all dogs were recorded without anesthesia by the connection of a cannula from a gauge 18 needle in the femoral artery directly to a mercury manometer. The readings (always taken in the morning) were made every 2 or 3 days for a period of 3 weeks before operation, to establish control blood pressure values. The experimental hydronephrosis was produced in the trained animals; blood pressure readings were then taken at bimonthly intervals as the animals developed chronic or progressive hydronephrosis. Ten animals from the chronic group were observed over a period of ±6 months, and 10 from the progressive group over a period of 2 to 6 months, or until death from acute uremia.

Table I shows blood pressures for 4 dogs in the chronic nonuremic group (those not included did not differ essentially from those reported). These pressures, it will be noted, differed only within experimental limits of error from the control pressures.

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[4] Hartwick, A., *Z. f. d. ges. Exp. Med.*, 1930, **69**, 462.

[5] Harrison, T. R., Mason, M. F., Resnik, H., and Rainey, J., *Trans. Assn. Am. Phys.*, 1936, **101**, 281.

[6] Eichelberger, L., *J. Urology*, 1938, **40**, 366.

TABLE I.
Blood Pressures in Hydronephrotic Dogs.

	Mean Blood Pressure—mm. Hg														
	Range of 10 control readings	2 weeks after partial constriction	Weeks After Partial Constriction Plus Opposite Nephrectomy												
			2	3	4	6	8	10	12	14	16	18	20	22	24
			Chronic Hydronephrosis												
Dog F	120-130	130	130		132		130		125	130	125	125	125		
Dog J	118-125	125	122		118		125		122	125	125	120	120		125
Dog B	120-135	135	130		139		132		130	132	135	135	130		135
Dog D	120-130	125	122		128		125		127	127	125	120	128		122
			Progressive Hydronephrosis												
Dog O	130-140	130	128		140	138	140	138 140* 170† 175‡							
Dog C	120-130	130	150	140	130	172P	128		130	130	160P	135	130	130* 150† 180‡	
Dog E	125-135	140	130		140	140	172P		135	135	140	125	165P	137	135
Dog T	120-130	130	135		135	135	130 135† 180‡								

P following a reading indicates the animal was in pain.
* Three days before death.
† Two days before death.
‡ Day of death; N.P.N., mg%—Dog O, 200; Dog C, 250; Dog T, 205.

Table I also shows the blood pressures for 4 representative animals from the progressive group. In this group also, the mean blood pressures did not differ from the control pressures, except in the following instances, which cannot be considered as direct results of the decrease in amount of functioning renal tissue *per se:* (1) If, during the progress of the hydronephrosis, blood pressure readings were taken at a time when the sac of the hydronephrotic kidney was distended and the animal was sensitive to handling and pressure, a moderate elevation of blood pressure might be found. This was interpreted as the result of pain, since when readings were taken at a time when the sac was less full, and the animal was entirely comfortable, control values were found. (2) In a few animals, during the last few days of life a rise in blood pressure was found to accompany the severe uremia (non-protein nitrogen in excess of 200 mg %; serum phosphate 4.0 mM; marked acidosis); this was considered comparable to the rise found clinically in previously non-hypertensive patients when in acute uremia.

These results would indicate that dogs with only one kidney, in which a considerable portion of the renal tissue has been destroyed by intraureteral pressure, maintain a normal mean blood pressure.

Presumably in these hydronephrotic kidneys the increased pressure occurring in the kidney pelvis may not be great enough to reduce the blood flow in the kidney sufficiently to produce hypertension on the basis of ischemia. Hence, these findings are quite compatible with Goldblatt's conclusive demonstration that reduction of the blood flow to the functioning components of the kidney causes the development of hypertension.[7] It should be noted, too, that infection was absent, or present to only a minor degree, in the hydronephrotic kidneys. Renal vascular inflammatory changes were, therefore, not a factor, in contrast to their rôle in human pathology.

Summary. 1. Mean blood pressures were recorded on dogs at bimonthly intervals as they developed chronic or progressive hydronephrosis. 2. The data indicate that dogs with only one kidney, in which a considerable portion of the renal tissue has been destroyed by intraureteral pressure resulting from partial constriction of the ureter, maintain a mean blood pressure within normal limits.

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[7] Goldblatt, H., Lynch, J., Hanzal, R. F., and Summerville, W. W., *J. Exp. Med.*, 1934, **59**, 347.

10866

Colloidal Sulphur to Modify Extirpation Diabetes.

HARRY GREENGARD AND GARR J. BURT. (Introduced by A. C. Ivy.)
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There have been several reports in the literature stating that sulphur administration will increase sugar tolerance in the normal and the diabetic organism. Bürgi and Gordonoff[1] stated that the glycogen content of the livers of sulphur-fed rabbits was 2 to 3 times the normal level. According to Földes[2] the administration of sulphur to human diabetics caused in some cases, a fall in blood sugar, increased sugar tolerance, and elimination of glycosuria and ketonuria. Penetti[3] obtained a 15-20% lowering of the blood sugar in urethanized rabbits by subcutaneous injections of colloidal sulphur in doses of 30-40 mg per kilo. Daniel and Popescu-Buzeu[4] reported that sulphur-containing mineral water markedly lowered the blood sugar in dogs and in normal and diabetic humans. Koyasako[5] and Yoshikawa[6] found that the daily application of 15% sulphur salve to the skin of rabbits did not affect the blood sugar level, but did increase the sugar tolerance and likewise increased insulin hypoglycemia and attenuated adrenalin hyperglycemia.

On the other hand Foucin and Sandor[7] found daily injections of sulphur to be entirely without effect in diabetes and attributed any reduction in blood sugar which it caused as due to colloid shock; and Lewis and Lewis[8] observed no alteration in glycogen content of the livers of rats fed sulphur for several weeks.

Since none of the experiments cited above provided conclusive evidence, the problem was reinvestigated by us, using depancreatized dogs which had been standardized over many months as to diet, insulin dosage, and glucose output in the urine. To these dogs was administered a colloidal sulphur preparation of proven absorbability known to be non-toxic in ordinary doses.

The 3 dogs used were maintained on their standard diet throughout the 7-week period in which observations were made. They were fed and given the required dose of insulin daily at 9:00 a.m. and 5:00 p.m. The urine was collected under toluene and was measured and analyzed for glucose daily. Control analyses were performed for 7-day periods, alternating with 14-day periods in which 5 cc or 10 cc (250 or 500 mg) of colloidal sulphur was given daily by stomach tube.

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[1] Bürgi and Gordonoff, *Klin. Wchnschr.*, 1926, **5**, 466.

[2] Földes, *Z. f. d. Gesamte Exp. Med.*, 1927, **55**, 615.

[3] Penetti, *Z. f. d. Gesamte Exp. Med.*, 1927, **57**, 584.

[4] Daniel and Popescu-Buzeu, *C. r. soc. biol.*, 1931, **106**, 106.

[5] Koyasako, *Folia endocrinol. Japan*, 1931, **6**, 2.

[6] Yoshikawa, *Sei.-i-Kwei Med. J.*, 1934, **53**, 74.

[7] Foucin and Sandor, *C. r. soc. biol.*, 1926, **96**, 697.

[8] Lewis and Lewis, *J. Biol. Chem.*, 1927, **74**, 515.

Results. The average daily glucose outputs are shown in Table I.

TABLE I.

Dog No.	1st control period (7 days)	1st sulphur period (250 mg and 14 days)	2nd control period (7 days)	2nd sulphur period (500 mg and 14 days)	3rd control period (7 days)
I	2.76	0.87	1.22	2.64	1.47
II	5.44	2.76	3.82	3.27	1.63
III	10.52	8.31	12.13	10.81	5.21

It is evident from the above data that the administration of colloidal sulphur neither aggravated nor ameliorated the diabetic state of the animals; the glucose outputs were unchanged except for the variations ordinarily encountered. At no time was a sugar-free specimen obtained.

Conclusion. The administration of colloidal sulphur in daily doses of 250 or 500 mg has no effect on the diabetic state of the depancreatized dog.

10867

Tachyphylaxis to Renin.

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In the course of experiments on the preparation, purification, and assay of kidney extracts containing "renin", the phenomenon of tolerance or "tachyphylaxis" was noted. By this is meant the progressive decrease in response occurring as a result of the repeated intravenous injections of equal amounts of pressor material at equal intervals into an assay animal so prepared as to record blood pressure.

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[*] Florsheim Fellow.

This phenomenon has been noted by other observers.[1-11] In most instances they only comment on its occurrence or show protocols indicating its presence but provide no data. Bingel and Strauss,[2] however, suggest a means of avoiding the phenomenon.

Early in our investigation of the pressor activity of fresh kidney extracts many different preparations were used. Included among these were crude saline extracts, the products of isoelectric precipitation at various pH values, and of dialysis, ultrafiltration, and adsorption procedures. From the many animals used in testing these preparations, 21 were observed which were injected with equivalent amounts of active pressor material at various intervals. Fourteen of this group showed tachyphylaxis, 2 were doubtful and 5 showed none. The nature of the preparation did not seem to have any bearing on the appearance of tachyphylaxis. Because of the observations of other workers, our own experiences, and particularly because of the frequent need to make repeated injections into animals in the assay of active preparations, it was felt that the phenomenon should be investigated. In particular we desired to answer the following questions: (a) Does acetone used in the preparation of the "renin" give a product which causes tachyphylaxis? That is a question raised in a personal communication from Dr. Wakerlin. (b) Does the type of anesthesia of the assay animal affect the phenomenon? (c) Is a species difference between the source animal and the assay animal a factor? (d) Is the time interval between successive injections a significant variable?

Equal amounts of a standard "renin" preparation were injected intravenously into dogs whose carotid arteries were connected to a recording mercury manometer. The animals were anesthetized in various ways as indicated in the tables. An outline of the method of preparing the "renin" used follows: Fresh hog kidneys were frozen in carbon dioxide snow, crushed, extracted with 4 volumes of 5% sodium chloride for several hours, and filtered. The filtrate was

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[2] Bingel, A., and Strauss, E., *Deutsch. Arch. f. klin. Med.*, 1909, **96**, 476.

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[7] Pearce, R. M., *J. Exp. Med.*, 1909, **11**, 430.

[8] Pickering, G. W., and Printzmetal, M., *Clin. Sci.*, 1938, **3**, 211.

[9] Shaw, H. B., *Lancet*, 1906, **1**, 1295.

[10] Thauer, R., *Cent. fur Inn. Med.*, 1934, **54**, 2.

[11] Tigerstedt, R., and Bergmann, P. G., *Skand. Arch. f. Physiol.*, 1898, **8**, 223.

TABLE I.

	Rise in blood pressure, mm Hg		
	1st Inj.	2nd Inj.	3rd Inj.
Results obtained with acetone-treated and non-acetone-treated preparations. Injections at 30- to 60-minute intervals. Morphine sulphate and ether anesthesia.			
Acetone-treated preparations (10 dogs)	33	19	11
Non-acetone-treated preparations (5 dogs)	25	10	9
TABLE II.			
Results obtained with various types of anesthesia. Injections at 30- to 60-minute intervals.			
Morphine sulphate and ether (15 dogs)	30	16	10
Nembutal (7 dogs)	27	16	12
Sodium barbital (2 dogs)	41	13	5
Urethane (1 dog)	45	20	4
No anesthesia—animals with pithed brain and cord (3 dogs)	46	13	7
TABLE III.			
Results obtained with extracts from different species injected into assay animals of the same and of different species. Injections at intervals of 30 to 60 minutes. Morphine sulphate and ether anesthesia.			
Dog preparations into dogs (4 dogs)	22	8	6
Hog ” ” ” (12 ”)	32	20	11
TABLE IV.			
Results obtained with variation in the time interval between successive injections. Anesthetics indicated.			
Less than 1 hr intervals (11 dogs) (6*, 5†)	32	19	15
1 hr intervals (11 dogs) (9,* 2†)	26	12	10
2 ” ” (10 ”) (4,* 6†)	30	19	21
3 ” ” (10 ”) (5,* 5†)	27	30	34
Individual results obtained in dogs injected at 3 hr int.			
Dog No. — Anesthesia			
1 — Nembutal	23	22	—
2 — ”	66	39	60
3 — ”	20	31	36
4 — ”	28	26	30
5 — ”	43	64	55
6 — Morphine sulphate and ether	15	18	31
7 — ” ” ” ”	28	40	42
8 — ” ” ” ”	16	7	14
9 — ” ” ” ”	16	41	16
10 — ” ” ” ”	17	7	23

* Morphine sulphate and ether.
† Nembutal.

saturated with slight excess of sodium chloride, overlayed with toluene, incubated at 37°C for 24 hours, and filtered. The precipitate† was resuspended in 4 volumes of distilled water, dialyzed in a cellophane bag against tap water until the sodium chloride content of the suspension was 3.5%, and filtered. The filtrate was acidified with hydrochloric acid to a pH of 3.5 (glass electrode) and filtered. The filtrate was mixed thoroughly with kaolin in amounts to make a 10% suspension, and filtered. The kaolin was eluted with 1% disodium hydrogen phosphate, the pH of the mixture being adjusted to 7.5 and filtered. The filtrate was treated in the cold with 7 to 10 volumes of acetone and filtered. The precipitate was dissolved immediately in small amounts of distilled water, overlayed with toluol and kept in the refrigerator. A similar concentrate of dog kidneys was prepared except that in the original 5% saline extraction, freshly minced dog kidneys were substituted for the frozen tissue. The kaolin eluate was injected and compared with the kaolin eluate of hog kidney (Table III).

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† This precipitate was furnished through the courtesy of Dr. David Klein of Wilson Laboratories.

The standard "renin" preparation described is such that the injection of an amount containing 0.4 mg per kilo (30 gamma nitrogen content) produces a rise of approximately 30 mm of mercury in a dog anesthetized with morphine-ether. Approximately 300 g of fresh hog kidney yield 1 g of dried active substance.

Of the factors above noted, the only one which seems to have any significance pertaining to the appearance of tachyphylaxis is the time interval between successive injections. Study of the data in Table IV indicates that as the interval between the injections is increased, tachyphylaxis becomes less marked. In the dogs injected at 2-hour intervals, some tolerance is apparent, whereas, in the dogs injected at 3-hour intervals, a suggestion of some augmentation is evident. The importance of the time interval was recognized by Bingel and Strauss[2] who, injecting a press juice made from rabbit kidneys into a rabbit under urethane anesthesia, report that tolerance is abolished by waiting 2 hours between injections.

Conclusions. 1. A method is described which will give a reliable, relatively stable, active "renin" preparation. 2. The method of preparing the "renin", the use of acetone in its preparation, the type of anesthesia used for the assay animal, species difference between source and assay animal, do not appear to affect significantly the appearance of the phenomenon of "tachyphylaxis" or tolerance. 3. The time interval between successive injections does significantly affect the appearance of tachyphylaxis, and the effect is more pronounced the shorter the interval.[2] The effect is least noticeable when the interval is 120-180 minutes. 4. Assay procedures carried out in dogs where the interval between successive injections is less than 2 hours are misleading unless the phenomenon of tolerance is taken into consideration.

10868 P

Cerebral Arteriography in the Dog and in Man with a Rapidly Excreted Organic Iodide.

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Up to the present cerebral arteriography has been carried out by the intracarotid injection of colloidal thorium dioxide.[1, 2] The objections and potential dangers inherent in the use of this drug are well known.[3, 4, 5]

Recently Robb and Steinberg[6] have used Diodrast (3,4-diiodo-4-pyridon-N-acetic acid diethanolamine) in a 70% solution by intravenous injection for visualization of the chambers of the heart, pulmonary circulation, and great blood vessels in man. They demonstrated the excellence of 70% Diodrast as a radiographic agent for this purpose. The absence of severe reactions and the complete and rapid excretion of this substance, which they reported, led us to study its possibilities for demonstration of the cerebral circulation radiographically.

In 7 dogs the common carotid artery was exposed under intraperitoneal sodium pentobarbital anesthesia. In 5 dogs 30 cc of a warmed 70% Diodrast solution was injected intracarotidly and X-rays of the skull taken during the injection. Excellent visualization of the arteries of the injected side of the brain was obtained. In one dog 30 cc of 35% and in another 30 cc of 50% Diodrast solution were used. Visualization of the cerebral circulation with the 2 lower concentrations was not adequate for clinical use. One animal to which a large overdose of the hypnotic had been given by error failed to recover from the anesthesia. The other dogs upon recovering from the anesthetic seemed to suffer no ill effects whatever. Two dogs were sacrificed at the 24th hour post injection and the brains removed. Grossly no abnormalities were observed. Preliminary histological examinations were likewise negative. Two more animals were sacrificed after 5 days and upon removal the brains appeared

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[1] Moniz, Egas, *L'Angiographie Cerebrale*, Masson et Cie, Paris, 1934.

[2] Turnbull, F., *Am. J. Roent. and Rad. Ther.*, 1939, **41**, 166.

[3] Northfield, D. W. C., and Russel, D. S., *Lancet*, 1937, **1**, 377.

[4] Editorial, *J. A. M. A.*, 1937, **108**, 1656.

[5] Biskind, M., *Drug and Therapeutic Survey*, 1938, **1**, 21.

[6] Robb, G. P., and Steinberg, I., *Am. J. Roent. and Rad. Ther.*, 1939, **41**, 1.

grossly normal. The remaining dogs after 4 weeks appeared perfectly normal as to body weights and in actions and habits.

Following this experience in animals, 25 cc of 70% Diodrast solution was injected into the common carotid artery of each of 3 men. A transient reaction was noted during the injection in each case which was characterized by flushing of the skin, slight nausea and a temporary elevation of the systolic blood pressure by 20 to 34 mm. This was followed by moderate sweating and then slight headache lasting 2 or 3 hours. An X-ray film taken during the injection clearly visualized the cerebral arteries as illustrated in Fig. 1.

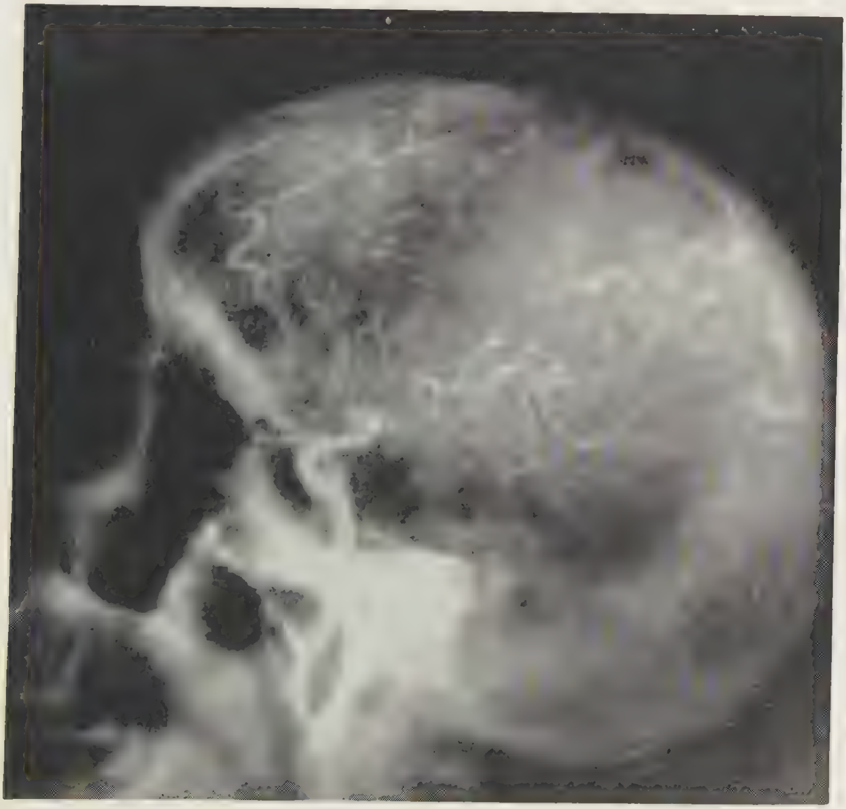

Fig. 1.
Normal arteriogram obtained with 70% Diodrast.*

Summary. The excellent results obtained in visualization of the cerebral arteries in dogs and man by the intracarotid injection of 70% Diodrast solution prompt us to recommend this method when such visualization is indicated.

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* Radiographer, Dr. George Plehn, City Hospital, Welfare Island, N. Y.

10869

Tests of Mammalian Gonad-Stimulating Hormones on Gonads of Fishes.

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Houssay[1] implanted pituitary glands of fish and induced a prompt ovulation of the nearly ripe eggs of another species of fish, and Cardoso[2] and von Ihering[3] confirmed this result and further showed that extracts and suspensions of fish pituitary stimulate growth in testes and ovaries of various species of fish. Azevedo and Canale[4] reported that piscine pituitaries are effective while extracts from mammalian and avian pituitaries are not effective for gonad stimulation in fishes. Koch and Scheuring[5] injected prolan into 3- to 4-year-old rainbow trout with negative results. Matthews[6] obtained practically no stimulation of the testes of Fundulus with an unfractionated extract of sheep pituitaries, but demonstrated that removal of the pituitary in adult *Fundulus* is followed by inactivity or atrophy of the gonads. On the other hand, Boucher[7] and associates report slight evidence for the hastening of sexual maturity in eels by small amounts of pregnancy urine, while Calvet[8] and Damas[9] report success in stimulating the ovaries of lampreys with both prolan and whole extract of mammalian pituitary. It is evident that further studies are needed to resolve the question whether vertebrates higher than fishes have evolved a new and distinct gonad-stimulating hormone (FSH) or whether an apparent refractoriness of piscine gonads to mammalian FSH perhaps rests upon a basis hitherto unexplored.

We have tested the action of 3 types of previously assayed mammalian FSH preparations on 90 rainbow trout *(Salmo shasta?)* aged 11 and 16 months without any transfer of the fish from their normal habitat. For these trout and facilities for maintaining them under wholly normal conditions during our tests we are indebted to Mr. Stanley C. Walters, Foreman, New York State Fish Hatchery, Cold Spring Harbor, N. Y. A brief abstract of these results was published.[10]

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[1] Houssay, B. A., *Rev. Soc. Argent. Biol.*, 1930, **6**, 686.

[2] Cardoso, D. M., *Compt. Rend. Soc. Biol.*, 1934, **115**, 1347.

[3] von Ihering, R., *Zool. Anz.*, 1935, **111**, 273.

[4] Azevedo, P., and Canale, L., *Arg. Inst. Biol.* (Sao Paulo), 1938, **9**, 165.

[5] Koch, W., and Scheuring, L., *Zool. Anz.*, 1936, **116**, 62.

[6] Matthews, S. A., *Biol. Bull.*, 1939, **76**, 241.

[7] Boucher, S., Boucher, M., and Fontaine, M., *Compt. Rend. Soc. Biol.*, 1938, **116**, 1284.

[8] Calvet, J., *Compt. Rend. Soc. Biol.*, 1932, **109**, 595.

[9] Damas, H., *Bull. Soc. Roy. des Sci. de Liège*, 1933, No. 4, 94.

In November, 60 11-month trout (weight about 30 g), divided into 4 equal groups, were injected with NaCl (control), pituitary FSH No. 429, mare serum powder, and prolan (Elberfeld). No. 429 was free from prolactin, was relatively rich in thyrotropin and contained little adrenotropin; a dose of 1 mg daily for 4 days gave a 400% increase in testes of young doves (weight 150 g), and in 20 mg daily doses for 3 days into 21-day rats gave ovaries of 48 mg, uteri of 55 mg, testes of 376 mg and seminal vesicles of 14.8 mg. The prolan contained 15 R.U. per mg. The mare serum powder, when administered in 10 mg doses daily for 4 days to young doves, increased testis weight by 300%. During the first 11 days the daily dosage per fish was as follows: No. 429, 0.5 mg; prolan, 2 mg; mare serum powder, 8 mg. During a final 10-day period all of these daily dosages were doubled. These quantities were injected intramuscularly in 0.2 cc of fluid.

Immediately upon first handling and injection of these small trout one or more males in all groups released sperm (milt) before injection, and a similar release of sperm was observed on many other days during the 21-day period of injection. Two such males were among the control and 8 among the 3 injected groups. At the end of the period of injection (or at death) 3 of 11 male control had testes filled with sperm, 3 such males were among the 7 males injected with No. 429, 3 among the 8 injected with prolan, and 3 among the 7 injected with mare serum. In addition, 3 treated males showed only a few sperm. Altogether 10 males injected with gonad-stimulating preparations had minute thread-like testes with no sperm and no developmental stage beyond spermatocytes undergoing their early period of growth.

Ovaries of injected females showed no detectable stimulation though ovaries of control and all treated groups contained oöcytes entering the growth period. There were some instances of oöcytes beginning to form yolk but no heavily yolked eggs were found. In view of the stage of ovarian development thus observed—a stage which might be considered especially sensitive to gonad-stimulating hormone—the definitely negative result of all these tests is especially notable.

In the following April, 30 rainbow trout of the same brood (then aged 16 mo, and slightly larger than the 11-mo group) were divided into 3 equal groups and injected daily for 15 days with NaCl (control), 4 mg prolan (15 R.U. per mg) or 1 mg FSH No. 474. This latter preparation, in doses of 1 mg daily for 4 days into 450 g pigeons, increased testis weight by 125%. Three fish of each group were injected intraperitoneally, the others intramuscularly. At autopsy the testes of the 7 treated males (only one with sperm) did not differ significantly from the 4 male controls. The ovaries of 13 treated fish were also insignificantly different from those of 6 control in both histology and in weight (control, 40.5 g; prolan, 39.8 g; No. 474, 44g).

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[10] Johnson, M. W., and Riddle, O., *Year Book, Carn. Inst. of Wash.*, 1936, **35**, 53.

It is notable that at the N. Y. State Hatchery where our fish were treated (and maintained at 51°F) these rainbow trout breed (at and after 2-years old) in November, not in the following March-April as they frequently do in some other localities.

Summary. (1) FSH from beef pituitaries, powdered serum of the pregnant mare, and a commercial preparation of human pregnancy urine (Prolan, Elberfeld) were otherwise proved potent and then injected daily for 21 or 15 days into 65 rainbow trout *(Salmo shasta?)* aged 11 or 16 months. An additional group of 25 trout of the same brood served as control (NaCl injected), and all fish were maintained in their normal habitat at 51°F while under test. (2) A fraction of the males had sperm-producing testes before injection but an equal or greater number had unstimulated minute thread-like testes at the end of treatment. The largest oöcytes in the ovaries of all groups were beginning to form yolk but no heavily yolked eggs were present. (3) Neither of the potent mammalian gonad-stimulating preparations used measurably stimulated either testes or ovaries in these trout. These results support the view that the FSH elaborated by pituitaries of fish differs qualitatively from that of mammals but much further study of the problem is needed.

10870

Separation of Lipids in Gravimetric Acetone Method for Plasma Total Protein.

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In the method of Bierry and Vivarro for the estimation of plasma total protein as modified by Guillaumin, Wahl and Laurencin, proteins are precipitated by adding 3 ml of plasma to 10 ml of acetone, centrifuging and washing the precipitate twice with acetone. Commenting on this method, Peters and van Slyke[1] state, "This is the most rigidly exact of plasma protein methods, because the acetone treatment removes lipoids. . . . In the other methods . . . the lipoids are precipitated with the proteins, and their nitrogen content probably causes a slight plus error in the protein figure."

Boyd[2] has shown that when cold, fat solvents are used to extract plasma lipids, a minimal initial volume of solvent is required per volume of plasma if complete extraction is to be obtained. With Bloor's extracting fluid, 3:1 alcohol-ether which is generally conceded to be the best lipid extractor, a minimum of 20 volumes of solvent per one volume of plasma were found necessary. On checking the protein method of Bierry and Vivarro as outlined by Peters and van Slyke,[1] it was found, as was to be expected, that lipids are not completely removed by the acetone. A few typical examples are given in Table I to show that lipid values in alcohol-ether extracts by Boyd's method[2] are appreciably and consistently higher than lipid values of the combined acetone mother liquors in the protein method.

The acetone protein method does not, therefore, remove all the lipids and the presumption is that the portion left is weighed later with the proteins. In plasma with normal amounts of protein and lipids, this would result in a plus error of 2 to 3% for the protein fraction. In the plasma of patients with nephrosis, the error would be much greater. I have encountered total lipid values as high as 2,000 mg % in nephrotic patients and assuming such occurred along with a true plasma protein value of say 2%, and assuming the same relative extraction of lipids (it might easily be lower in such cases), the plus error for protein could be as high as 25%.

On the other hand, if protein were estimated as nitrogen, the error

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[1] Peters, J. P., and Van Slyke, D. D., *Quantitative Clinical Chemistry*, Vol. II, p. 688, 1932.

[2] Boyd, E. M., *J. Biol. Chem.*, 1936, **114**, 223.

TABLE I.
A Comparison of Lipids Found by Boyd's Extraction and in the Acetone Mother Liquors of Human Plasmas.
(Values are expressed in mg %.)

Sample	Total Lipid		Total Cholesterol		Phospholipid	
	Boyd	Acetone	Boyd	Acetone	Boyd	Acetone
1	527	378	145	71	149	133
2	471	323	123	72	136	113
3	642	498	159	114	193	167
4	528	392	135	94	159	155

would be much less—that is, using the acetone method but estimating the nitrogen of the precipitate rather than its weight. Lipid nitrogen is contained in the phospholipid fraction. It represents but a small part of the phospholipid fraction. The phospholipid fraction represents but one quarter of the total lipid. And phospholipids are more readily extracted by moist acetone (in the presence of other lipids and without magnesium chloride) than other lipids such as cholesterol.

The protein method of Bierry and Vivarro may be suitably modified to remove all of the lipids by simply diluting the plasma in a volume of acetone corresponding to the proportion of alcohol-ether used by Boyd.[2] It has been found that when plasma is diluted in 20 or 25 volumes of acetone, the acetone extract contains amounts of lipids identical to those in the alcohol-ether extract of the same sample of plasma. The suggested modification of the Bierry and Vivarro method as outlined by Peters and van Slyke[1] is thus to add 3 ml of plasma to 75 ml of acetone instead of 10 ml in the initial step of the procedure.

10871

Physiological Contraction of Double Hearts in Rabbit Embryos.

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The purpose of this paper is to show that the primitive lateral hearts in rabbit embryos are physiologically active and independent for some hours before they fuse into a single heart.

Knowledge concerning the early development of the mammalian heart has been obtained, for the most part, by the study of fixed preparations. However, during recent years, several investigators have observed and reported the action of the hearts in living embryos in tissue culture.[1-4]

Embryos, the ages of which were calculated from observed matings, were obtained from a series of 12 rabbits. At the desired time the rabbit was given a moderate dose of nembutal, surgical anesthesia obtained by ether as needed, and the uterus removed. Embryos were cultured in hanging drops of adult rat plasma and 15-17-day rat embryo extract. A few embryos were observed immediately in buffered salt solution (Tyrode) before they were dissected away from the uterine wall.

Attempts to predict the stage of development of the embryo at a given time were only partially successful; about 50% of the rabbits were consistent, whereas the remaining 50% showed variation. However, 8 day 16 hour embryos were usually in the 4 or 5 somite stage. The hearts of these embryos were well formed and showed evidence of having been active for some hours. The 2 beating hearts were entirely independent of each other and were more widely separated that those observed in the rat by Goss.[3-4] The contraction originated at the venous end and both hearts beat at a regular rate, but the left was about 10 beats per minute faster than the right. The rate of the left heart varied from 47-49 beats per minute in the 3 somite stage, to about 80 per minute at the time of fusion of the 2 hearts (Table 1). The rate was correlated with the stage of development. In the earlier stages the ventricle was the only part of the heart involved in the contraction. A short time before the 2 hearts fused the atrium also showed contractions, in which case the impulse originated in the atrium.

Not only were the hearts beating at independent rates, but the type of contraction was surprisingly characteristic of each heart. The left side, which tends to be saccular, has a simple rapid "snap" contraction which originates in the venous end of the heart. The right heart tends to be of a tubular form, and the contraction is more complex. It may be described as a twisting wringing motion, similar to the 'peristaltoid' contraction described by Patten[5] in the chick. The time required to complete this contraction is longer than that of the left, so that the latent period between contractions is more nearly equal than one would expect from their respective rates.

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[1] Waddington, C. H., and Waterman, A. J., *J. Anat.*, 1933, **67**, 355.

[2] Nicholas, J. S., and Rudnick, D., *Proc. Nat. Acad. Sci.*, 1934, **20**, 656.

[3] Goss, C. M., *J. Exp. Zool.*, 1935, **72**, 33.

[4] Goss, C. M., *Anat. Rec.*, 1938, **70**, 505.

[5] Patten, B. M., and Kramer, T. C., *Am. J. Anat.*, 1933, **53**, 349.

TABLE I.
Heart Rates in Cultured Rabbit Embryos.

No.	Age of embryo	No. of somites	Hours in culture	Heart rate		Remarks
				Left	Right	
W11B	8 days	3	2	49	37	Hearts widely
	12 hr		4	51	40	separated
			10	56	44	
G6A	8 days	4	2½	57	48	Hearts separated
	16 hr		4	58	49	” ”
			10	60	48	” ”
W12A	8 days	5	1½	66	38	” ”
	15 hr		3½	65	38	” ”
			9	68	43	” ”
W10B	8 days	7	1½	82	79	” ”
	15 hr		3½	82	80	” ”
			9	63	63	” united
W10A	8 days	8	2	92	78	” separated
	15 hr		4	94	76	” ”
			10	65	65	” united
Br8D	8 days	9	2½	86	78	” separated
	17 hr		5	89	77	” ”
Br8E	8 days	11	3	88		Single heart
	17 hr		5	86		” ”
			10	88		” ”
Br8C	8 days	12	3	113		” ”
	17 hr		5	86		” ”

A backward and forward motion of cellular elements suspended in the plasma within the lumen of the vitelline veins was regularly observed in 7 somite embryos, and was occasionally seen at earlier stages. Both the vitelline veins and the aortae were connected to the hearts of their own side.

A similar movement has also been noted in the aortae at a slightly later stage in development. This movement is a pulsation caused by the contraction of the hearts, and though present on both sides, is more marked on the right side as would be expected because of the more vigorous type of contraction. There is a suggestion of approaching establishment of circulation, since the cellular elements do not always return to their original position within the aorta.

Two embryos developed single hearts after 10 hours in culture; these were 7 and an 8 somite embryos in which the hearts were widely separated when first observed. It was noted that in these hearts the impulse originated in the left atrium, as is the case in embryos with single hearts at the time of removal from the uterus.

The embryos were kept at a constant temperature of 38°, both during and between observations. They were not handled for one or 2 hours after being cultured, and then as little as possible. Recorded observations were made only after the culture had rested on the stage of the microscope for a few minutes. A few embryos have been observed in buffered salt solution (Tyrode) before removal from the uterine wall. These controls compared favorably with the observations made later in culture.

Summary. Rabbit embryos, removed during the ninth day of gestation, were cultured in hanging drops. Studies of the heart show that the 2 primitive lateral heart tubes beat regularly for some hours before they fuse to form one heart. There are marked differences between the 2 sides both morphologically and physiologically. The hearts develop considerably before fusing, as evidenced by the movement of cellular elements within the vessels. At early stages the contractions involve only the ventricles, but before fusion to form a single heart the impulses may be seen to originate in the respective atria.

10872

Andromimetic Effect of Estrogen upon the Clitoris of the Rat.*

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During the past 2 years, a number of female rats from an inbred strain of unrecorded origin have been found to possess spontaneous ovarian tumors which were invariably associated with defective external genitalia[1]. In order to interpret the physiology of these pathological ovaries, attempts were made to duplicate the abnormal external genitalia by the administration of sex hormones to normal females at different periods of postnatal life. It was observed that the genitalia, characteristic of the tumorous strain, could be experimentally produced by the injection of estrogen during the first week of postnatal life, but not if the treatments were begun thereafter. Since the studies on the ovarian tumor will be published subsequently, the present paper will be devoted to an interpretation of the effects of estrogen upon the external genitalia of the normal juvenile female rat.

It has been reported[2, 3] that the administration of estrogen to the pregnant rat, during the terminal stages of gestation, produced a characteristic modification of the clitorine prominence in the female offspring. Similar effects were observed when large amounts of estrogen were injected during the first few days of postnatal life.[3] Previous workers[2, 3] interpreted this abnormality as a hypotrophic effect of the estrogen which resulted in the absorption of the tissue which embryonically formed the anterior wall of the urogenital sinus. For this reason, the condition has been referred to as a "hypospadias." [3, 4]

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* The estrogen (Theelin) was supplied by Dr. Oliver Kamm, Parke, Davis and Company.

[1] Turner, C. Donnell, *Anat. Rec.*, 1939, **73**, 75.

[2] Hain, A. M., *Quart. J. Exp. Physiol.*, 1935, **25**, 131.

[3] Greene, R. R., Proc. Soc. Exp. Biol. and Med., 1937, **36**, 503.

TABLE I.
Postnatal Administration of Estrogen to Juvenile Rats.

Litter number	No. of treated animals	IU estrogen at each injection	No. of injections	Age in days at beginning of treatment	Age in days at autopsy
S1	1	100	60	2	120
S2	2	100	45	1	88
T1	4	100	17	20	118
S3	2	100	9	1	131
T2	2	50	10	1	65
T3	2	50	5	1	92
T4	3	100	20	10	146

Our observations are based upon 16 treated animals (7 litters) of the Wistar strain. The estrogenic treatments were begun at different ages and were continued for varying periods of time (Table I). The most pronounced effects were obtained when several large doses of estrogen were administered during the first few days after birth, the animal receiving no further treatment (litters S3, T2, T3). In these animals, the clitorine prominence is deeply cleft along the line of the raphe between the prominence and the vaginal orifice (Fig. 3). In the fissure, anterior to the urethral orifice, is a conspicuous teat-like elevation, the glans clitoridis. A slender frenulum extends caudally from the base of the glans. Small erectile bodies differentiate from the tissue between the glans and the urethral orifice (Fig. 3). When the estrogen is administered continuously from birth until postnatal life (litter S1), the grooved condition of the clitorine prominence is not so pronounced but slight hypertrophy of the glans clitoridis and the differentiation of small erectile bodies does occur (Fig. 2). At this writing, we are unable to explain why estrogen administered constantly from birth until postpuberal life has less influence upon the clitorine prominence than estrogen administered only for a short period subsequent to birth. The clitoris of the newborn rat seems to be particularly sensitive to estrogen since these treatments do not

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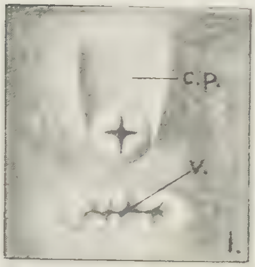
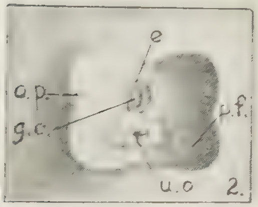
[4] Greene, R. R., and Ivy, A. C., *Science*, 1937, **86**, 200.





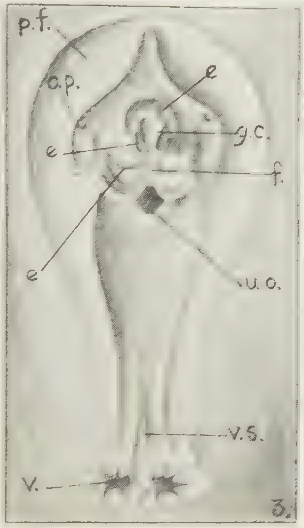


FIG. 1.

The external genitalia of a normal adult female rat. c.p., clitorine prominence; v., vaginal orifice.

FIG. 2.

An end view of the clitorine prominence of a rat (litter S1) which had received estrogen continuously from the second day of postnatal life until autopsy at 120 days of age. The preputial folds (p.f.) are separated in order to reveal the glans clitoridis (g.c.), erectile tissue (e.), urethral orifice (u.o.), and orifices of the preputial glands (o.p.).

FIG. 3.

The abnormal external genitalia of a rat (litter S3) which received estrogen only during the first eighteen days of postnatal life. The animal was autopsied at 131 days of age. Compare with Figs. 1 and 2. e., erectile bodies; f., frenulum: g.c., glans clitoridis; o.p., orifice of preputial gland; p.f., preputial fold: u.o., urethral orifice; v., vaginal orifice; v.s., vaginal septum.

produce grossly abnormal external genitalia when begun after the tenth day of postnatal life.

In the normal female rat, there is only very slight cleavage of the preputial fold (Fig. 1). The glans clitoridis of the normal adult is a very small protuberance situated anterior to the urethral orifice. The tissue around the base of the glans is heavily vascularized, and slight indication of erectile bodies may be observed occasionally. The glans clitoridis, erectile tissue and urethral meatus are surrounded by the invaginated preputial epithelium. The orifices of the preputial glands are located along the inner margin of the preputial fold and, in the adult smegma may be expressed from these pores.

Evidence of urinary incontinence was observed in only 2 of the 9 animals which received estrogen during the first week subsequent to birth. Previous investigators associated this functional impairment with improper differentiation of the urinary sphincter[2, 3] and regarded it as "hypospadias" resulting from a hypotrophic effect of the hormone.[3] We believe that these abnormal external genitalia may be interpreted best on the basis of an andromimetic effect of the estrogen. The extensive fissure in the clitorine prominence apparently results from more extensive cleavage of the preputial fold than occurs normally. The estrogen also produces a slight enlargement of the clitoris and a feeble differentiation of erectile tissue. In affected animals, the urethral orifice is more posterior to the glans clitoridis than normally. This probably results from the separation of the preputial epithelium from the wall of the distal urethra and from the hypertrophy of the erectile tissue between the glans and the urethral meatus. If this is the correct explanation of the wide separation of the glans clitoridis and the urethral orifice in the treated animals, we question the justification of terming this defect a "hypospadias."

Summary. The administration of estrogen to female rats during the first week of postnatal life produces a defect in the external genital organs characterized by cleavage of the preputial fold, hypertrophy of the glans clitoridis, and a feeble differentiation of erectile tissues. Since these changes resemble, except for extent, those produced by postnatal androgen, it is concluded that estrogen exerts a mild andromimetic effect upon the clitoris of the rat.

10873

Distribution and Excretion of Sulfapyridine in the Guinea Pig.

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Since the guinea pig is almost exclusively used for experimental tuberculosis therapeutic studies on sulfonamide compounds, it is important to secure information on the distribution and excretion of the most potent anti-mycobacterial sulfapyridine compound in this animal[1]. Our early *in vitro* and *in vivo* studies of the bacteriostatic action of sulfanilamide and "prontosil soluble" on mammalian, avian, and paratubercular acid-fast bacteria[2], confirmed the findings of

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[1] Birkhaug, K. E., *Brit. Med. J.*, 1939, **2**, 54 (Review of literature).

[2] Birkhaug, K. E., *Beretninger, Chr. Michelsen Institute*, 1939, **9**, 3.

Marshall, Emerson, and Cutting,[3] Proom and Buttle,[4] Whitby,[5] Fuller,[6] and Stewart, Rourke, and Allen,[7] and others, that sulfanilamide is distributed in all the liquids and tissues in the animal organism, more or less proportionally with the water content of the liquids and tissues.

The present study deals with the rate of absorption, distribution and excretion of free and conjugated sulfapyridine in the guinea pig after the oral administration of 0.2 g per kg of the drug suspended in 0.5 cc of a 5% acacia solution. The animal was placed in a small wire basket within a porcelain basin in order to collect urine. A group of 40 normal guinea pigs, having an average weight of 436 g, were employed. At the end of 30 minutes, and 1, 2, 3, 4, 5, 6, 7, 8, 12, 24, 48 and 72 hours, an animal was sacrificed with ether. Thus at each stated time interval 3 animals were killed and the average results recorded. Determinations of free and conjugated sulfapyridine were done of the urine voided and removed from the bladder, contents of the stomach, duodenum, jejunum, ileum, colon and rectum, bile, blood, liver, lungs, spleen, pancreas, kidneys, adrenals, testes, muscles of the heart, legs, abdomen and chest, bone-marrow, eyes, spinal cord, brain, skin, and fat, as well as of milk, and of fetal blood.

The methods for estimation of free and total sulfapyridine consist of modifications of the methods suggested by Proom,[8] and Marshall and Litchfield.[9] One millilitre of liquid or one g of tissue (the latter finely macerated with kieselguhr) is diluted with 2 ml distilled water; one ml of 20% aqueous solution of trichloracetic acid is then added, and the mixture filtered through a small filter-paper. To one ml filtrate is added 0.05 ml (one drop) of 0.5% sodium nitrite solution. After standing for 3 minutes to diazotize, 0.1 ml of a 1 M sodium dihydrogen phosphate solution containing 0.5% of ammonium sulfamate ($NaH_2PO_4.H_2O$, 13.8 g; ammonium sulfamate, 0.5 g water, 100 cc) is added in order to destroy excess nitrous acid which forms after diazotization, and to buffer the solution before coupling with dimethyl-a-naphthylamine. After standing for 2 minutes, 0.5 ml of a 1.0% alcoholic solution of dimethyl-a-naphthylamine is also added, and the whole is mixed by gentle shaking. After standing for 15 minutes to allow the color to reach its maximum intensity, the color is compared with that obtained by treating in a similar manner standard solutions containing known quantities of sulfanilamide. To determine the total sulfanilamide, one ml of the filtrate is treated with 0.1 ml of 2 N HC1, heated in a boiling water bath for one hour, cooled, and the volume adjusted to one cc. The subsequent procedure is the same as for the estimation of free sulfanilamide. Since sulfanilamide standards were used for comparison, these were converted to sulfapyridine concentrations by multiplying by the factors of 1.25 in the free and 1.5 in the total sulfapyridine determinations, according to the experimental findings of Schmidt and Hughes.

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[3] Marshall, E. K., Jr., Emerson, K., Jr., and Cutting, *J. A. M. A.*, 1937, **108**, 953; *J. Pharm.*, 1937, **61**, 196; *J. A. M. A.*, 1938, **110**, 252.

[4] Proom, H., and Buttle, G. A. H., *Lancet*, 1937, **1**, 661.

[5] Whitby, L. E. H., *Ibid.*, 1937, **1**, 1517.

[6] Fuller, A. T., *Ibid.*, 1937, **1**, 194.

[7] Stewart, J. D., Rourke, G. M., and Allen, J. G., *J. A. M. A.*, 1938, **110**, 1885.

[8] Proom, H., *Lancet*, 1938, **1**, 260.

[9] Marshall, E. K., Jr., and Litchfield, J. T., *Science*, 1938, **88**, 85.

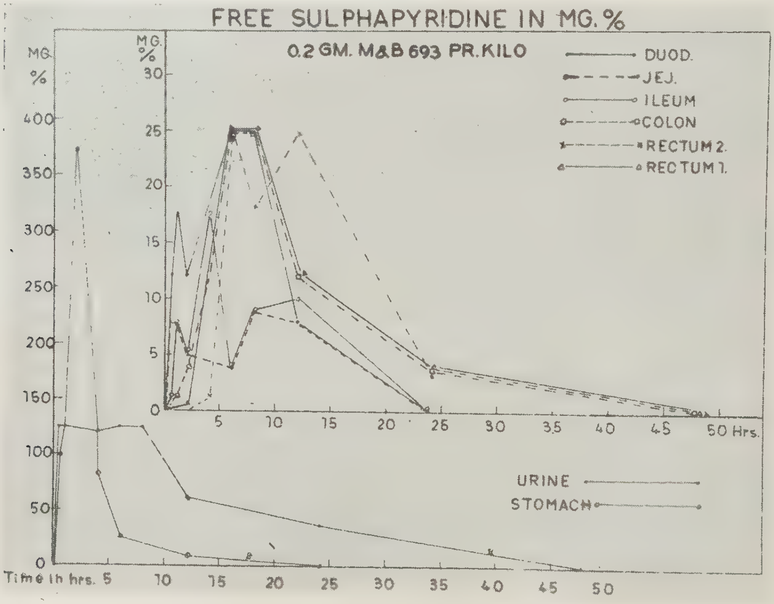

FIG. 1.

Graph 1 shows that of the 200 mg sulfapyridine ingested per kg, 45.7% remain in the stomach after 30 minutes; 33.5% after one hour; 30.25% after 2 hours; 18.75% after 3 hours; 7.1% after 4 hours, and only 1.9% after 12 hours. Apparently the absorption from the stomach is nearly complete in 4 hours after the oral administration of the drug. Very little of the drug passes into the smaller gut (1.08% in one hour; 1.51% in 4 hours, and 0.86% is recovered in 8 hours). It is possible that resorption of the drug takes place from the mucosa in the large gut since the contents in the colon showed 12 mg % of free sulfapyridine after 4 hours, 25 mg % after 8 hours, and 3.7 mg % after 24 hours. Likewise, the lower segment of rectum showed 22 mg % after 4 hours, 21 mg % after 8 hours, and 3.7 mg % after 24 hours. Approximately 25% of the total sulfapyridine in the gastro-intestinal tract is in the conjugated form.

The maximum urinary excretion of sulfapyridine takes place during the first 8 hours when approximately 50% of the ingested drug is excreted. After 24 hours about 72% of the drug is eliminated in the urine, and less than 1% of the administered drug is excreted between 24 and 48 hours. Presumably is the difference eliminated in the feces. Approximately 30% of the total sulfapyridine excreted in the urine is in the conjugated form.

Graph 2 shows that the bile concentration of free sulfapyridine reaches to 18 mg % and the conjugated form to 2 mg % after one hour, and 25 mg % of the free and 2 mg % of the conjugated form in 4 hours. After 12 hours the curve of free sulfapyridine in the bile declines to 5 mg % and after 24 hours to 0.12 mg %. The blood concentration curve of free sulfapyridine rises sharply after one hour to 10 mg %, while the conjugated form amounts to 3 mg %. The maximum concentration occurs 4 hours after ingestion and amounts to 12 mg % of free and 3 mg % of the conjugated form. After 12 hours the curve of free sulfapyridine declines to 3 mg % and after 24 hours to 0.12 mg %. By estimating that the blood equals 8% of the guinea pig body weight, we observe that of the ingested 0.2 g sulfapyridine per kg, 6.25% is found in the blood after one hour, 7.6% after 4 hours, 5.1% after 8 hours and 0.08% after 24 hours.

Graphs 2, 3 and 4 show the distribution of free sulfapyridine in liquids, organs and tissues. It is quite apparent that the drug is distributed throughout the entire body and that the period of maximum concentration coincides with that in the blood, namely—between the fourth and seventh hour after the ingestion of the drug. The highest concentration curves occur in the bile, blood, skeletal and heart muscles, liver, lungs, kidneys and testes in descending strength in the order mentioned (25-11 mg %). Then follows the salivary glands, pancreas, and eyes with concentrations varying between 4.5 and 2.7 mg %. The lowest concentrations are found in the adrenal glands, bone-marrow, skin and fat (2.0 to 1.2 mg %). Approximately 10-20% of the total sulfapyridine in the above-mentioned liquids, organs and tissues occurs in the conjugated form.

The milk concentration curve was studied in 2 guinea pigs. It was found to coincide with the concentration of free and conjugated sulfapyridine in the blood. The fetal blood concentration curve is available at 8, 18, 24 and 48 hours after the maternal ingestion of

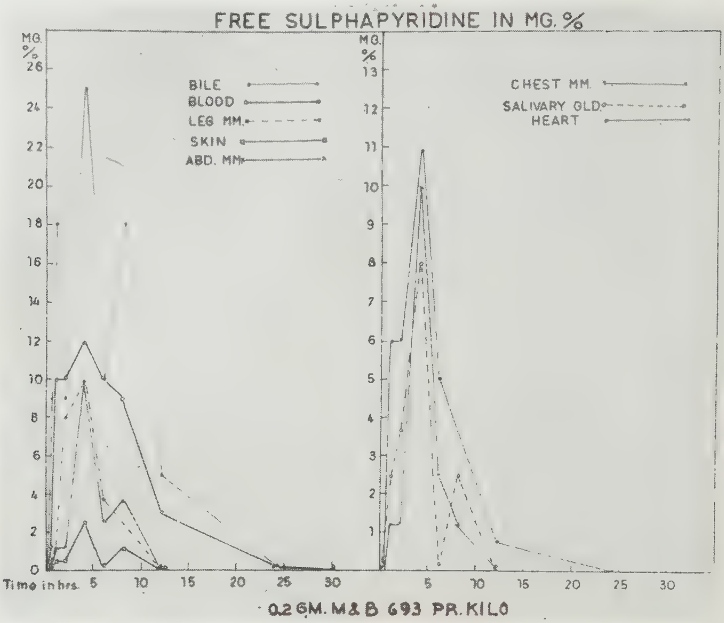
FREE SULPHAPYRIDINE IN MG.%
MG. %
26
24
22
20
18
16
14
12
10
8
6
4
2
0
BILE
BLOOD
LEG MM.
SKIN
ABD. MM.
Time in hrs. 5 10 15 20 25 30
MG. %
13
12
11
10
9
8
7
6
5
4
3
2
1
CHEST MM.
SALIVARY GLD.
HEART
5 10 15 20 25 30
0.2 GM. M.& B 693 PR. KILO

Fig. 2.

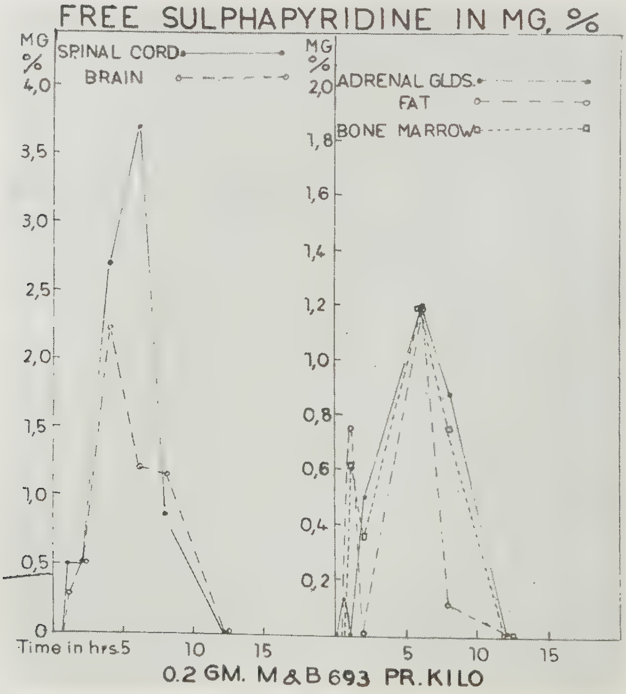
FREE SULPHAPYRIDINE IN MG. %
MG %
4,0
3,5
3,0
2,5
2,0
1,5
1,0
0,5
0
SPINAL CORD
BRAIN
Time in hrs. 5 10 15
MG %
2,0
1,8
1,6
1,4
1,2
1,0
0,8
0,6
0,4
0,2
ADRENAL GLDS.
FAT
BONE MARROW
5 10 15
0.2 GM. M & B 693 PR. KILO

Fig. 3.

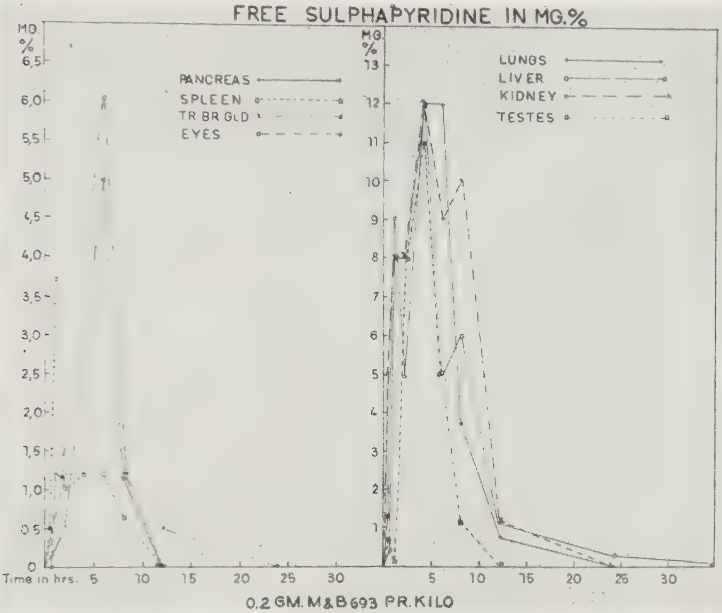

Fig. 4.

0.2 g of the drug per kg, and this is nearly identical with the maternal blood concentrations of free and conjugated sulfapyridine.

Conclusion. The orally administered sulfapyridine is nearly completely absorbed from the stomach after 4 hours, and is distributed to every liquid, tissue and organ in the guinea-pig body, mostly in the free form. About 70% of the ingested drug is excreted in the urine after 36 hours and resorption from the larger gut mucosa may account for the discharge of the balance with the feces.

10874

Bacteriostatic Effect of Sulfapyridine, Sulfanilamide and Prontosil Rubrum *in vitro* on Mycobacteria.

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In view of the inhibitory action of certain sulfanilamide compounds on the development of an infection of guinea pigs with human and bovine tubercle bacilli,[1] the present study was undertaken to secure more exact information on the *in vitro* effect of sulfapyridine, sulfanilamide, and prontosil rubrum on mammalian, avian and certain paratubercular strains of mycobacteria. Carpenter and Gibbons[2] have reported that sulfanilamide and disulon in 1:2,500 to 1:10,000 concentrations either completely or partially inhibited the growth of chromogenic mycobacteria while no such bacteriostatic effect was noted on bovine and human tubercle bacilli. Rist[3] found that sulfanilamide (1162 F) in 200 mg % concentration completely inhibited the film formation of bovine and human strains of tubercle bacilli while 20 mg % concentration only partially delayed growth of these strains. An avian strain which grew in the depth of the tube was completely inhibited in a 20 mg % concentration of sulfanilamide (1162 F). However, the compound 1358 F (4-4′-diaminophenylsulphone) exerted the same bacteriostatic effect in 10 times weaker concentrations than the 1162 F compound. Rist, Bloch and Hamon[4] extended the study *in vivo* and found that both these sulphonamide compounds inhibited the multiplication of virulent avian tubercle bacilli in rabbits and guinea pigs. Birkhaug[1] reported a similar *in vivo* bacteriostatic effect of prontosil soluble on virulent bovine tubercle bacilli in the guinea pig.

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[1] Birkhaug, K. E., *Brit. Med. J.*, 1939, **2**, 54 (Review of literature).

In our study 14 strains of mycobacteria were employed, of which 4 were bovine, 3 of each were human and avian tubercle bacilli, all of considerable virulence, and 4 paratubercular cultures: *M. johnei, M. piscium, M. Boquet and M. Vallée.* Concentrations of 10, 50 and 90 mg % of sulfapyridine, sulfanilamide and prontosil rubrum (4-sulphamido-2, 4-diaminoazo benzol) were made in Sauton's synthetic medium adjusted to pH 7.4. The solutions were made at 100°C and held at 38°C to avoid recrystallization. Ten cc of each concentration was placed in 2.5x20 cm cotton-plugged sterile tubes. Similar amounts of Sauton's medium were used for controls. The 14 strains of mycobacteria were previously grown on Sauton's medium for 3 weeks and all had formed surface films. A 3 mm loopful of film (approximately one mg dry weight) from each strain was floated on the surface of the liquid in each tube. A rubber cap was placed above the cotton plug to prevent evaporation. These cultures were left in a slanting position in the incubator for 7 weeks at 37.5°C. At that time they were autoclaved and filtered through previously weighed small filter papers. After being completely dried at 90°C until the weight became constant, the filter papers were again weighed and the difference between the first and second weights was recorded as bacterial growth. The experiment was repeated twice and the averages of these 3 trials are shown in Table I.

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[2] Carpenter, C. M., and Gibbons, L. L., *J. Bacteriol.*, 1939, **37**, 226.

[3] Rist, N., *C. R. Soc. de Biol.*, 1939, **130**, 972.

[4] Rist, N., Bloch, F., and Hamon, V., *Ibid.*, 1939, **130**, 976.

TABLE I.

Bacteriostatic Action of sulfapyridine, Sulfanilamide, and Prontosil rubrum on Mammalian, Avian, and Paratubercular Acid-fast Bacilli.

Dry weight of bacterial growth (mg) in 10 cc of Sauton's liquid medium containing 10, 50, and 90 mg% conc. of the sulphonamide compounds, after 7 weeks' incubation at 37.5°C.

	Sulfapyridine			Sulfanilamide			Prontosil rubrum			Control
				Mg% concentrations						
Strain	10	50	90	10	50	90	10	50	90	Control
Human R-51	56	29	16	96	60	51	72	49	14	107
" H-37	67	30	19	101	71	61	61	42	12	103
" S-46	38	12	0	128	67	30	21	0	0	130
Bovine Ep.-2	86	48	23	154	93	45	89	13	10	152
" VR-12	39	17	11	112	77	46	132	15	11	130
" T5080	84	62	11	121	72	51	66	0	0	118
" T3396	51	0	0	104	82	47	55	0	0	109
Avian B-44	50	0	0	111	104	102	19	0	0	115
" NR-61	62	0	0	117	101	98	21	0	0	119
" NR-62	18	0	0	111	78	70	57	0	0	108
Myc. johnei I	71	64	34	163	155	142	117	0	0	165
Myc. piscium	50	32	0	104	63	42	83	55	28	109
Myc. paratub. Boquet	18	10	0	134	87	49	0	0	0	142
Myc. paratub. Vallée	63	10	0	104	64	41	45	0	0	104
Avg wt mg	54	23	8	119	84	63	60	13	5	122
Statistics: t	9.0	13	19	0.45	4.6	6.1	5.5	15	21	
vs. Control P	0.01	0.01	0.01	>0.9	0.01	0.01	0.01	0.01	0.01	

By summing up the average weights for each concentration of the 3 sulphonamide compounds and by analyzing the difference between the mean weights of the control and each sulphonamide concentration by the statistical method suggested by Fisher,[5] it is quite apparent that both sulfapyridine and prontosil rubrum in 10 mg % concentration are capable of producing a significant inhibition of mycobacterial growth which sulfanilamide is not able to do before the concentration is elevated to 50 mg %. But even at that concentration the inhibition of growth is only 32% less than the control weight. Not before the concentration of sulfanilamide reaches 90 mg % is the inhibition of mycobacterial growth comparable with that produced in 10 mg % concentrations of sulfapyridine and prontosil rubrum.

Conclusion. Sulfapyridine and prontosil rubrum possess more potent anti-mycobacterial properties *in vitro* than sulfanilamide.

The author is greatly indebted to Messrs. May and Baker for the supply of sulfapyridine (M&B 693), and to the I. G. Farbenindustrie A. G. "Bayer" for the supply of prontosil rubrum.

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[5] Fisher, A. R., *Statistical Methods for Research Workers,* Oliver and Boyd, London, 1936, pp. 128-166.

10875

Effect of Cooling on the Mechanism of Insulin Action.

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The experiments reported here are concerned with the effects of lethal doses of insulin on the survival period of cooled and non-cooled rabbits. Some observations on the effect of temperature on insulin action are also reported.

1. *The Body Temperature Lowering Action of Insulin.* Forty-eight-hour fasted rabbits were used. Blood sugars were determined by the Folin-Wu method. In order to produce a lowering of body temperature without using drugs or keeping the animal in an ice bath, the rabbits were wetted with 95% alcohol and placed in front of a fan. Using this method, the rate of heat loss was increased and the body temperature usually fell 2-7° C., reaching a maximum in 90-150 minutes. In the normal animal this increase in heat loss was compensated by an increase in heat production so that when the animal was not re-wetted, the temperature returned to normal in 1-3 hours. If, however, large doses of insulin were given at the time of the initial wetting, the mechanism of heat production was interfered with and the temperature drop was not only greater in the first 2 hours but continued to fall until it reached a point a few degrees above that of the room, and remained at that level from 6-18 hours without the need of any further wetting. Prevention of the blood sugar fall by the administration of glucose with the insulin prevented this hypothermic action of the insulin. Table I summarizes the results of these 3 groups.

TABLE I.
Effect of Insulin and Insulin and Glucose on Body Temperature of Alcohol Wetted Rabbits.

	Hours						
	0	1	2	3	4	5	6
No insulin, Avg 5 animals	39.1	36.0	35.9	36.9	38.5	39.1	39.0
3 u./kg., Avg 6 animals	38.9	34.3	32.3	27.7	29.4	28.6	29.3
3 u./kg + 5 g glucose Avg 4 animals	39.3	38.4	38.1	38.7	39.1	39.2	39.2

2. *Duration of Insulin Action in Cooled Rabbits.* Twelve rabbits were cooled by repeated wettings with alcohol and when the temperature reached 25-28°, 0.5 units of insulin per kg were given intraven-

ously. Eight rabbits, not previously cooled but given the same dose of insulin, served as controls. The duration of insulin action (as evidenced from the blood sugar) was greatly prolonged in the cooled animals, returning to normal after 6-10 hours, as compared with 3-5 hours for the non-cooled. Nevertheless, the rate of lowering the blood sugar was about the same in the 2 groups.

3. *Survival Period of Cooled Hypoglycemic Rabbits.* Eight fasted rabbits were given massive doses of insulin and their temperatures were kept at normal levels. Of these animals, 6 died from 3-6 hours after the onset of convulsions, while 2 recovered spontaneously from the hypoglycemia at the end of 7½ hours.

Thirteen rabbits were cooled to temperatures between 25-30°C and given 2 injections of insulin (6 units/kg each injection) 8 hours apart. Seven rabbits lived 14 hours, 3 lived 18 hours, 1 lived 20 hours, 1 lived 26 hours and 1 rabbit was maintained for 148 hours, with blood sugars ranging from 25-45 mg %. The insulin in the latter rabbit was reduced after the second day to 3 units twice daily. The respiration and heart rate of the cooled animals were irregular and reduced.

Discussion and Conclusions. 1. The results on the hypothermic action of insulin are in line with the observations of Dworkin and Finney,[1] who found that when a woodchuck was given enough insulin to produce hypoglycemia, it lost its temperature control and could readily be put into a state of "artificial hibernation." We also confirm their observation that the administration of glucose to cooled hypoglycemic animals resulted in the rise of their body temperature.

The results reported here give support to a thesis that available glucose is essential for the maintenance of normal body temperature.

2. Insulin hypoglycemia is prolonged by cooling the animal. Whether this is due to a reduction in the rate of gluconeogenesis (as a result of cooling), or due to a prolongation of insulin action cannot be definitely established at this time.

3. The survival period of rabbits receiving lethal doses of insulin is prolonged by cooling to temperatures below 30° C. It has been established that cooling to temperatures below 30°C greatly reduces the rate of metabolism,[2] as well as inhibiting or stopping insulin convulsions.[3]

The hypothesis is offered that both the increase in the survival period and the inhibition of convulsions in cooled animals receiving massive doses of insulin are a result of the decreased metabolic rate and the subsequent decreased demand for foodstuffs by the vital centers of the brain.

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[1] Dworkin, S., and Finney, W. H., *Am. J. Physiol.*, 1927, **80**, 75.

[2] Finney, W. H., Dworkin, S., and Cassidy, G. J., *Am. J. Physiol.*, 1927, **80**, 301.

[3] Cassidy, G. J., Dworkin, S., and Finney, W. H., *Am. J. Physiol.*, 1925, **73**, 417.

10876

Choline Esterase Activity in Various Portions of the Rabbit Heart.

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The concentration of acetylcholine in the auricle is approximately 10 times that in the ventricle.[1-3] Gaddum has pointed out that tissues rich in acetylcholine contain greater quantities of choline esterase.[4] It was considered of interest to determine whether the latter relationship also holds for various portions of the heart, in which case credence would be lent to the possibility that this enzyme plays the rôle of a defense mechanism in the vagus regulation of the heart.

Twelve normal rabbits weighing between 1500 and 2200 g were utilized for these experiments. The animals were sacrificed by injecting air into the marginal ear vein; the chest was then opened, and the heart immediately extirpated. The individual auricles and ventricles and the interventricular septum were dissected out. The interauricular septum was included with the right auricle as was also the mouth of the superior vena cava. The valves and their attachments were discarded. The membranous septum was included with the interventricular septum. The tissue was freed from superficial blood, weighed, and then macerated in a mortar with purified and ignited sand. Bicarbonate-Ringer solution was added in the proportion of 10 cc per gram of tissue. The mixture was centrifuged and choline esterase measurements were made with ½ cc of the supernatant fluid and 1½ cc of substrate solution (acetylcholine chloride dissolved in bicarbonate-Ringer, 5 mg/cc). The enzyme activity was determined by the manometric method using the War-

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[1] Witanowski, W. R., *Arch. ges. Physiol.*, 1925, **208**, 694.

[2] Plattner, F., *Ibid.*, 1926, **214**, 112.

[3] Engelhart, E., *Ibid.*, 1930, **225**, 721.

[4] Gaddum, J. H., *Gefässerweiternde Stoffe der Gewebe*, 1936, 75, George Thieme, Leipzig.

TABLE I.

Date	No.	Left Auricle		Right Auricle		Left Ventricle		Right Ventricle		Septum	
		Wt, mg	Enz. activity*	Wt, mg	Enz. activity*	Wt, mg	Enz. activity*	Wt, mg	Enz. activity*	Wt, mg	Enz. activity*
9-20-38	1	372	58	225	58	907	12	430	22	340	25
9-22	2	245	134	175	67	1590	16	675	30	1240	26
9-23	3	270	94	315	85	1415	32	510	17	1263	28
9-27	4	174	88	168	74	1038	31	535	32	1195	18
10-13	5	234	63	248	62	1525	13	735	46	1690	23
2-23-39	6	242	60	273	64	1290	27	445	39	925	42
4-18	7	360	60	226	80	1485	—	725	9	1737	—
4-25	8	384	60	180	75	513	11	883	31	1132	34
7-7	9	190	103	147	87	996	6	465	25	990	6
7-10	10	352	49	132	52	1015	8	494	18	1105	6
7-13	11	147	99	287	50	1102	—	486	22	1212	—
7-19	12	353	102	271	90	1188	15	621	19	1214	8
Avg			81		70		17		26		22

* Enzyme activity expressed in mm³ CO_2 liberated in 1 hour at 30°C.

burg Apparatus at 30° in the same manner as previously employed.[5] The results of the measurements are given in the table. From this it can be seen that the auricles consistently contained a higher concentration of choline esterase than the ventricles. From the averages, it is apparent that the choline esterase activities fall in the following order: left auricle > right auricle > right ventricle > septum and left ventricle. Little difference exists between the latter 3, but the ventricular group is distinctly lower than the auricular. The enzyme concentration of the auricle was found to be 2 to 10 times that of the ventricle in the same heart. The average enzyme concentration in the whole auricle was about 3.5 times that of the entire ventricle including the interventricular septum.

The aforementioned investigations show that the auricle of the rabbit, which is rich in vagus nerve supply, contains a relatively high choline esterase concentration. Engelhart[3] found, biologically, that the rate of destruction of acetylcholine is faster in the auricle than in the ventricle. Plattner[2] showed that the auricle contains considerably more acetylcholine than the ventricle, a finding which was confirmed by Engelhart. The latter further showed that after stimulating the vagus nerve to the heart, there was a marked augmentation of the acetylcholine content in the auricle, but not in the ventricle. These results were even more exaggerated after physostigmine. From the data presented, it is apparent that a certain parallelism exists between acetylcholine and choline esterase concentrations. In view of this, and since the auricle has been found to be more sensitive to acetylcholine than the ventricle,[6, 7] the possibility may be considered that the choline esterase acts as a defense mechanism by destroying excess acetylcholine. In this manner exaggerated vagus effects due to the liberation of great quantities of "vagusstoff" would be obviated.

Summary. 1. The choline esterase concentration in the auricles of the rabbit heart was found to be considerably greater than that in the ventricles. 2. These data may be interpreted as supporting the viewpoint that there is an enzyme protective mechanism operating in the vagus system.

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[5] Glick, D., Lewin, A., and Antopol, W., PROC. SOC. EXP. BIOL. AND MED., 1939, **40**, 28.

[6] Lanczos, A., *Arch. ges. Physiol.*, 1930, **225**, 710.

[7] Anitschkow, S. V., *Arch. Exp. Path. Pharm.*, 1935, **177**, 260.

10877

Nutritional Achromotrichia.*

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Several reports have appeared in the literature to indicate that there is a factor in the vitamin B complex which is concerned with the maintenance of normal hair pigmentation in piebald or black rats. Its identity or non-identity with the known members of the vitamin B complex has not been definitely established.[1-4] Morgan *et al.*[1] and Lunde and Kringstad[2] have classified it as one of the "filtrate factors." Nicotinic acid and vitamin B_6 have been eliminated by Lunde and Kringstad. These workers also concluded from the lack of correlation between growth and achromotrichia that the chick antidermatitis factor was not concerned.[3] Morgan and Simms[4] found adrenal atrophy to occur simultaneously with the achromotrichia.

We became interested in this factor because of its possible relation to several rat factors being studied in this laboratory, namely, factor W,[5] pantothenic acid,[6] and the spectacled eye factor.[7] Thus far we have studied various concentrates of known potency in the above mentioned factors, using both the cure and prevention of achromotrichia as a test of their activity.

Piebald rats from our colony were used. The basal ration has the following composition: sucrose 67%, purified casein 18%, salts III[8] 4%, butter fat 9%, and cod liver oil† 2%. In addition each rat received 10 gamma thiamin, 20 gamma riboflavin, and 10 gamma vita-

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* Published with the approval of the Director of the Wisconsin Agricultural Experiment Station. Supported in part by a grant from the Research Funds of the University. We are indebted to the W.P.A. project No. 8649 for assistance in care of animals.

[1] Morgan, A. F., Cook, B. C., and Davidson, H. G., *J. Nutrition*, 1938, **15**, 27.

[2] Lunde, G., and Kringstad, H., *Avh. Norske Vid.-Akad. Oslo*, 1 Mat. Nat. Kl., 1938, Nr. 1.

[3] Lunde, G., and Kringstad, H., *Z. Physiol. Chem.*, 1939, **257**, 201.

[4] Morgan, A. F., and Simms, H. D., *Science*, 1939, **89**, 565.

[5] Elvehjem, C. A., Koehn, C. J., and Oleson, J. J., *J. Biol. Chem.*, 1936, **115**, 707.

[6] Oleson, J. J., Woolley, D. W., Elvehjem, C. A., and Hart, E. B., in press.

[7] Oleson, J. J., Bird, H. R., Elvehjem, C. A., and Hart, E. B., *J. Biol. Chem.*, 1939, **127**, 23.

[8] Arnold, A., and Elvehjem, C. A., *Am. J. Physiol.*, 1939, **126**, 289.

† The cod liver oil was provided by Dr. C. Nielsen, Abbott Laboratories, North Chicago, Illinois.

min B_6‡ per day. The achromotrichia usually developed within 6-10 weeks in the rats receiving the basal ration alone or with an inactive supplement. Some of our results are given in Table I.

TABLE I.
Effect of Various Liver Fractions on Nutritional Achromotrichia.

Liver fraction	Factor present in the concentrate	Activity
Liver extract	All factors	+
Fuller's earth filtrate	"Filtrate factors"	+
" " "	"Eluate factors"	—
Corn oil		—
Nicotinic acid		—
Adenylic acid		—
Vitamin B_6		—
Acid-ether extract	Pantothenic acid	—
$CHCl_3$ residue	" "	—
Barium salt	" "	—
Hexane butanol extract	Factor W	—
Acetone eluate	" "	—

Liver extract§ was very effective at a level of 250 mg/day. The fuller's earth filtrate was active when fed equivalent to the same level. The fuller's earth eluate, which we found would prevent paralysis and the hemorrhagic disease in rats,[7] was inactive when fed equivalent to 1 g of liver extract per day. Synthetic nicotinic acid, adenylic acid, and vitamin B_6 were completely without effect.

We have found that corn oil, which is known to prevent the symptoms of acrodynia in rats, will also prevent or cure the spectacled eye condition, which can be cured with the fuller's earth filtrate.[9] However 3-4 drops of corn oil per day had no effect on achromotrichia. This indicates that these two factors are unrelated.

Three different pantothenic acid concentrates were tested. The acid-ether extract and the chloroform residue fractions of Woolley *et al.*[10] and the barium salts preparation described in brief by Oleson *et al.*[6] were fed at levels equivalent to 1 g of liver extract per day for the first two named and 100 gamma pantothenic acid for the latter preparation. These preparations were not active in preventing the appearance of achromotrichia though the time of onset of the symptoms was delayed somewhat in most cases. This effect was not observed when the concentrate was treated with alkali to destroy the pantothenic acid. This indicates that the slight activity was destroyed along with the pantothenic acid by the alkali treatment.

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‡ We are indebted to Dr. Robertson, Merck and Company, Rahway, New Jersey, for the thiamin and vitamin B_6 used.

§ The liver extract was kindly supplied by Dr. David Klein, Wilson and Company, Chicago, Illinois.

[9] Oleson, J. J., Elvehjem, C. A., and Hart, E. B., unpublished data.

[10] Woolley, D. W., Waisman, H. A., Mickelsen, O., and Elvehjem, C. A., *J. Biol. Chem.*, 1938, **125**, 715.

Two preparations of factor W were tested at levels equivalent to 250 mg of liver extract per day. The first preparation was made by extracting liver extract thoroughly with dry butanol and then precipitating out considerable material by adding an equal volume of hexane.[11] The second, a much more purified preparation, was an "acetone eluate" preparation, kindly prepared for us by Mr. Simon Black.[11] Both of these preparations were inactive. The factor which prevents achromotrichia was undoubtedly lost in the early stages of the above preparations.

In confirmation of the results of Lunde and Kringstad[2] we have observed the "rusting" of the fur of albino rats fed the above and other purified diets and also have not been able to correlate the appearance of achromotrichia with the growth of the animals.

Thus it appears that the dietary factor which prevents nutritional achromotrichia is distinct from all factors of the vitamin B complex which have thus far been identified and associated with specific function in the nutrition of the rat. Further the factor does not seem to be involved in the growth of the rat.

10878 P

Hypersecretion of Gonadotropic Hormone of Pituitary Gland of Rats Resulting from Treatment with Antigonadotropic Serum.*

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Thompson and Cushing[1] have reported that the anterior pituitary gland of a dog chronically injected over a period of months with pituitary extract had an increase in the number of basophiles associated with an atrophy of the gonads. Collip, Selye and Williamson[2] also found similar changes in rats injected with pituitary gonadotropic extracts, and Severinghaus and Thompson[3] noted a corresponding atrophy of the gonads and basophilism of the anterior pituitary of dogs injected with an antigonadotropic serum. The increase in the basophile cells of the pituitary glands of these animals suggests there might be an increase in the gonadotropic hormone secretion in contrast to the atrophic condition of the gonads. Nevertheless no attempt has been made to determine whether or not the pituitary glands of such animals secrete an increased quantity of gonadotropic hormone.

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[11] Black, S., and Elvehjem, C. A., unpublished data.

* Supported in part by a grant-in-aid from the Wisconsin Alumni Research Foundation.

[1] Thompson, K. W., and Cushing, H., *Proc. Roy. Soc. B*, 1934, **115**, 88.

[2] Collip, J. B., Selye, H., and Williamson, J. E., *Endocrinology*, 1938, **23**, 279.

[3] Severinghaus, A. E., and Thompson, K. W., PROC. SOC. EXP. BIOL. AND MED., 1939, **40**, 627.

The work to be reported here is concerned with the effect upon endogenous secretion of the pituitary gonadotropic hormone in single and parabiotic rats following treatment with antigonadotropic serum.

The serum was obtained from rabbits which had been injected daily for a period of 2 months or longer with an aqueous extract of whole dried pituitary gland of sheep. Tests made with immature female rats showed that this serum can inhibit the action of gonadotropic hormone contained in the pituitary glands of human beings, sheep, horses, and rats. In a previous publication we have shown that such rabbit serum will also prevent the action of endogenous gonadotropic hormone secreted by the pituitary gland of a castrated rat in parabiosis with a normal female.[4]

Experimental Procedure and Results. 1. Twenty-four female rats were injected subcutaneously from the 10th to the 20th day of life with 0.5 cc per day or a total dose of 5 cc of antigonadotropic rabbit serum. After discontinuing the injections the day of vaginal opening of the treated rats was noted and all animals were killed 5 days after the vagina had opened. Littermate control rats were autopsied at the same age as the experimental animals. The average age of the treated rats at the time of vaginal opening was 28.5 days and the average ovarian weight 5 days later was 42 mg. The littermate control rats autopsied at the same age had an average ovarian weight of 16.1 mg. The ovaries of the experimental rats showed large cystic follicles, corpora lutea and blood points, whereas the ovaries of the control animals were juvenile in appearance.

2. Ten rats were injected from the 1st to the 11th day of life with 0.1 cc daily or 1 cc total dose of antigonadotropic rabbit serum. The vaginas of these rats opened at an average age of 21.9 days and the uteri were enlarged. The average weight of the ovaries of these animals was 16.7 mg. The ovaries of the untreated female littermates, which were killed at the same age as the experimental rats, averaged 14.2 mg. Thus, as a result of the treatment with the antigonadotropic rabbit serum the opening of the vagina was produced

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[4] Kupperman, H. S., Meyer, R. K., and Hertz, R., *Endocrinology,* 1939, **24**, 115.

23-28 days before the normal time for rats of our colony. In this experiment the ovaries of the treated rats were not significantly larger than the controls but the ovaries were stimulated as shown by the effect on the vagina and uterus.

3. Five male and 5 female rats were treated from the 17th to the 27th day of life with 0.5 cc antigonadotropic serum per day. At 31 days of age, 4 days after the injections were discontinued, the treated rats were placed in parabiosis, according to the technic of Meyer and Hertz,[5] with a littermate female in such a manner that the injected experimental rat was the right hand partner and the intact littermate female the left partner. It is to be emphasized that no other treatment was given these animals. The rats were autopsied on the 41st day of life after having been in parabiotic union for 10 days. In the female-female pairs the average ovarian weight for the left untreated female was 72 mg while the gonads of the right female which received the antigonadotropic serum before being united in parabiosis, averaged 41 mg. The ovarian weight of the left female partner in the 5 female-male pairs was 92 mg while the testes of the right partner averaged 982 mg. The average ovarian weight of 7 single littermate female rats autopsied on the 41st day of life was 17 mg. The average testicular weight of 8 single littermate male rats autopsied when 41 days old was 1344 mg.

The results obtained with the single and parabiotic rats indicate that following a short time treatment of rats with an antigonadotropic rabbit serum there is a hypersecretion of gonadotropic hormone from the pituitary gland of the treated rat. This effect is not the result of the presence of gonadotropic hormone in the serum of the rabbit since it causes atrophy of the gonads as long as the injections are given. The injection of 0.5 cc of normal female rabbit serum per day into 14 rats from the 10th to the 20th day of life did not accelerate or inhibit the development of the ovaries or hasten vaginal opening.

The explanation of the precocious development of the ovaries following the treatment with antigonadotropic serum is based in part on the fact that the serum prevents the action of the gonadotropic hormone produced by the pituitary gland of the rat. As a result the gonads receive little, if any, stimulation and consequently they produce very little, if any, gonadal hormone. The absence of the gonadal hormones results in a condition analogous to castration which causes the increase of gonadotropic hormone in the pituitary gland and the blood.[5] However, the increase in the gonadotropic hormone is rendered ineffective by the excess quantity of antigonadotropic serum injected and the gonads remain in an atrophied condition. When the antigonadotropic serum is discontinued the increased gonadotropic hormone being produced by the pituitary gland is not neutralized and as a result the gonads are stimulated to develop from an atrophied condition to one much greater than that found in normal animals of the same age. Further experiments are being made to determine whether the stimulation of the gonads resulting after the treatment with antigonadotropic serum is temporary or permanent.

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[5] Meyer, R. K., and Hertz, R., *Am. J. Physiol.*, 1937, **120**, 232.

Summary. Pretreatment of young female rats with antigonadotropic serum induced a hypersecretion of the gonadotropic hormone of the animal's pituitary. This effect was determined by the precocious development of the ovaries of the treated rats after discontinuing the injections, and by the ovarian hypertrophy occurring in female rats paired to pretreated male or female littermates. A theory is offered to explain the results obtained.

10879

On the Site of Formation of Citric Acid in the Dog.

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With the advent of methods of analysis suited to the determination of small amounts of citric acid,[1-3] interest in this compound and its salts as factors in intermediary metabolism has increased. The wide distribution of citric acid in the animal body has been indicated by Smith and Orten.[4] In addition, these authors[5] have shown that, in the dog following the rapid intravenous injection of the sodium salts of a series of organic acids, the output of citric acid in the urine is increased. This synthesis was shown to be particularly striking with malate, malonate, succinate, fumarate and maleate. When sodium malate was injected the maximum rate of excretion of citric acid occurred within 60 minutes after injection and returned to normal after 4½ to 6½ hours.[6]

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* Chas. Pfizer and Company Fellow, Yale University, 1936-37.

† Chas. Pfizer and Company Fellow, Wayne University College of Medicine, 1937-38.

[1] Thunberg, T., *Biochem. Z.*, 1929, **206**, 109.

[2] Kuyper, A. C., and Mattill, H. A., PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 863.

[3] Pucher, G. W., Sherman, C. C., and Vickery, H. B., *J. Biol. Chem.*, 1936, **113**, 235.

[4] Smith, A. H., and Orten, J. M., *J. Nutr.*, 1937, **13**, 601.

[5] Orten, J. M., and Smith, A. H., *J. Biol. Chem.*, 1937, **117**, 555.

Krebs, Salvin and Johnson[7] showed that 1-malic acid was changed to citric acid in the rat and recently Orten and Smith[8] demonstrated that the rat like the dog possesses the ability to form citric from dl-sodium malate. By means of comparative tissue analysis after intracardiac injection of sodium chloride into one group of animals and sodium malate into a second group, it was shown that renal tissue contained definitely more citric acid than did blood, muscle or liver. Similar analyses after bilateral nephrectomy led to the conclusion that "under the experimental conditions employed, the citric acid formed following the injection of sodium malate into rats either is produced chiefly in the kidney or is dependent upon the presence of the kidney for its formation."

A similar demonstration of the locus of formation of urinary citric acid from injected sodium malate has not been made in the dog. In view of the extreme rapidity with which the change has been shown to occur in this species, it was thought that, if the kidney is the site of this transformation, simultaneous analyses of the blood in the renal vein and in the femoral artery during the stage of most rapid formation of urinary citric acid, might reveal a difference in concentration of this substance attributable to "reverse leakage," so to speak, much as was found by Nash and Benedict for ammonia.[9]

In the present study 4 female dogs were used; in each animal one kidney was exteriorized in order to facilitate securing the samples of renal venous blood. Dogs A and S were prepared according to the method described by Rhoads[10] with the other kidney intact and in the normal site.‡ Dogs B and D were prepared in a generally similar way except that part of the 10th, 11th, and 12th ribs on the side of the transplanted kidney were removed to relieve tension. Also in Dogs B and D, the right kidney was removed after the other had been exteriorized, thereby restricting the synthesis of citric acid to the kidney, the blood of which could be analyzed. Ample time was allowed for the remaining kidney to assume the entire renal excretory function of the body. All of the dogs were given the citrate-low ration used previously by Orten and Smith[5] in such quantity that body weight was maintained.

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[6] Smith, A. H., and Orten, J. M., *J. Biol. Chem.*, 1938, **124**, 43.

[7] Krebs, H. A., Salvin, E., and Johnson, W. A., *Biochem. J.*, 1938, **32**, 113.

[8] Orten, J. M., and Smith, A. H., *J. Biol. Chem.*, 1939, **128**, 101.

[9] Nash, T. P., Jr., and Benedict, S. R., *J. Biol. Chem.*, 1921, **48**, 463.

[10] Rhoads, C. P., *Am. J. Physiol.*, 1934, **109**, 324.

‡ We are indebted to Dr. J. C. Hinsey of the Cornell University Medical School for the use of Dogs A and S. The experiments on them were carried out in the Laboratory of Physiological Chemistry, Yale University School of Medicine.

Citric acid was estimated directly on suitable aliquots of catheter specimens of urine and on trichloracetic acid filtrates of blood. The method of Pucher, Sherman and Vickery[3] was used, the final measurements being made in the early experiments with a Pulfrich spectrophotometer and in the later studies with a photoelectric colorimeter using a color filter with maximum absorption at 4250Å.

The plan of the experiments involved the simultaneous determination of citric acid in arterial and renal venous blood of unanesthetized dogs at the height of citric acid synthesis following the intravenous injection of dl-sodium malate. Five per cent dl-sodium malate in amounts providing 100 mg sodium per kg body weight was injected.

The blood samples were taken one-half hour after the injection in Dogs A and S and one hour after injection in Dogs B and D. All urine samples represented the one hour collection immediately following the injection. In Dogs A and S prior control experiments were carried out with sodium chloride in which 100 mg sodium per kg body weight in 2.5% solution was injected.

The results are summarized in Table I. It is clear that under the experimental conditions employed, there is little if any significant difference between the citric acid concentration in arterial blood and that in renal venous blood at the time when large amounts of this acid are being excreted in the urine. The data do not show that the kidney is contributing citric acid to the systemic circulation either under basal conditions (after the injection of sodium chloride) or when there is a rapid transformation of malic acid to citric acid

Although the results of the present study do not show that citric

TABLE I.

Dog	Treatment	Blood citric acid (mg%) Fem. Art.	Blood citric acid (mg%) Renal vein	Urine citric acid (mg/hr)
A	NaCl	3.0	2.7	1.4
S	,,	2.5	2.2	1.1
A	No malate	4.4	3.1	226.0
S	,,	2.5	2.0	156.0
B*	,,	1.4	1.4	98.0
D*	,,	1.5	1.3	75.0

* Unilateral nephrectomy after explantation of other kidney.

acid is formed in the kidney under the conditions of the experiment, they do not constitute evidence to the contrary. It remains for studies based upon a different experimental approach to demonstrate that in the dog, the kidney is concerned in the formation of citric acid from sodium malate administered intravenously. If the dog should prove to resemble the rat in this respect, the present study would indicate that the kidney is highly efficient, inasmuch as it removes the citric acid it has formed without returning a detectable amount to the systemic blood, even when one kidney is required to carry the entire burden (Dogs B and D) of synthesis and excretion.

The data might be interpreted as suggesting that citric acid may be formed elsewhere in the body. This point is supported by the work of Martensson[11] who worked with anesthetized fasting cats and found increases in citric acid in arterial blood over that in renal venous blood of magnitudes which may be significant. Furthermore, the gradual increase in the citric acid content of liver, blood and muscle after bilateral nephrectomy and injection of sodium chloride and sodium malate[8] also supports this point. The fact that Orten and Smith[8] state that "there *may* be an extra-renal source of citric acid" appears to have escaped the attention of Martius[12] who attributes to them the statement that citric acid originates only in the kidneys.

Summary. A comparison has been made of the citric acid content of renal venous blood with arterial (femoral) blood in dogs with an exteriorized kidney after the injection of dl-sodium malate. The venous blood was found to contain no more than or, perhaps, somewhat less citrate than arterial blood.

10880

Effect of Spinal Anesthesia on Intestinal Activity.

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Clinicians have long had the impression that spinal anesthesia causes increased intestinal contractions by release of the sympathetic inhibition of the intestines. This conception with the favorable results obtained in the treatment of megacolon and obstinate constipation by spinal anesthesia has been reviewed by Stabins, *et al.*[1] Markowitz and Campbell[2] noted the relief of experimental ileus following spinal anesthesia by directly observing the intestines after celiotomy or by observing changes in intestinal activity under the fluoroscope in animals under general anesthesia. Others[3] have reported the relief of ileus by splanchnic anesthesia in dogs anesthetized with barbital or ether. A more direct approach was the experimental one by Domenech Alsina[4, 5] whose work, however, was likewise performed on dogs under general anesthesia. Since different anesthetic agents were shown[6, 7, 8] to have different effects upon intestinal contractions it was deemed desirable to study the effect of spinal anesthesia upon the normal, unmedicated animal, *i. e.,* animals without previous medication and without general anesthesia. Accordingly, experiments were conducted on dogs previously prepared with Thiry-Vella fistulas of the jejunum and ileum.

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[11] Martensson, J., *Skand. Arch. Physiol.*, 1938, **88**, 303.

[12] Martius, C., *Angew. Chemie*, 1939, **52**, 223.

Six dogs weighing from 12 to 16 kg were used. Intestinal movements were recorded by inserting a balloon, connected to a water manometer as described by Jackson[9] into the lumen of the proximal end of the Thiry-Vella loop. No morphine or other medication was administered prior to the spinal injection. One hundred mg of procaine dissolved in 2 cc of normal saline was injected intrathecally in 4 to 5 seconds at the fifth or sixth lumbar interspace. By this technic anesthesia invariably occurred extending up to the fourth or sixth thoracic segment and lasting about an hour.

All the animals showed uniform results consisting of a marked increase of intestinal contractions (Fig. 1). This effect was maintained for at least 30 minutes after sensory and motor somatic activity had returned.

The result is to be expected since the innervation to the intestinal musculature by the sympathetic nervous system has an inhibitory action. There are convincing evidences to show that the same increase of tone and peristalsis also occurs in clinical spinal anesthesia.

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[1] Stabins, S. J., Morton, J. J., and Scott, W. J., *Am. J. Surg.*, 1935, **27**, 107.

[2] Markowitz, J., and Campbell, W. R., *Am. J. Physiol.*, 1927, **81**, 101.

[3] Ochsner, A., Gage, I. M., and Cutting, R. A., *J. A. M. A.*, 1928, **90**, 1847.

[4] Alsina, F. D., *Revista Med. de Barcelona*, 1927, **7**, 132.

[5] Alsina, F. D., *Presse Med.*, 1929, **37**, 66.

[6] Burstein, C. L., PROC. SOC. EXP. BIOL. AND MED., 1938, **38**, 530.

[7] Burstein, C. L., PROC. SOC. EXP. BIOL. AND MED., 1938, **39**, 396.

[8] Burstein, C. L., PROC. SOC. EXP. BIOL. AND MED., 1939, **40**, 122.

[9] Jackson, D. E., *Experimental Pharmacology*, C. V. Mosby, St. Louis, 1917, p. 278.

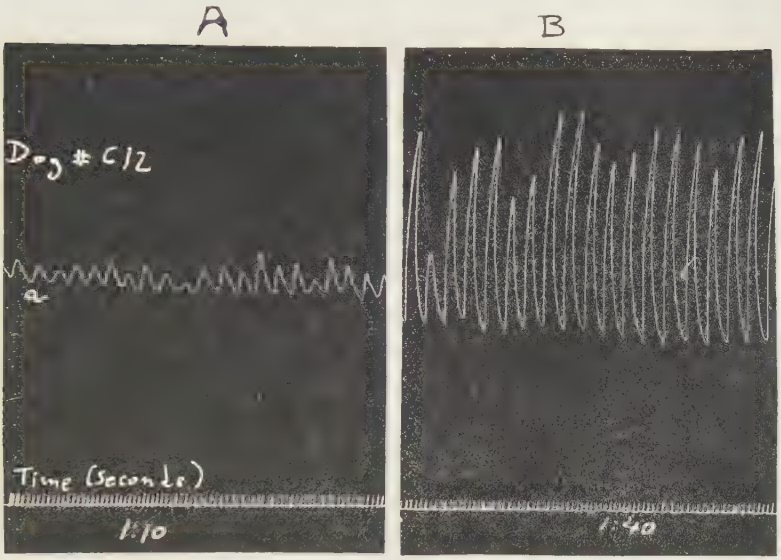

FIG. 1.
Effect of spinal anesthesia on intestinal activity of dog *in vivo*. Upper record (a), intestinal contractions. Time in second intervals. Spinal anesthesia was performed between A and B.

Surgeons have spoken of the "quiet belly" during this form of anesthesia due to the retraction of the abdominal contents. This increase in tone and peristalsis may lead to a rupture of a weakened segment of intestine, as actually happened in 2 cases of intestinal obstruction which were given spinal anesthesia at our clinic in Bellevue Hospital.

Conclusion. Spinal anesthesia causes an increase of intestinal contractions in the normal, unmedicated, dog.

10881

Uterine Effects from Single Treatments of Stilboestrol and Ethinyl-Estradiol in Monkeys.*

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This report deals with the bleeding interval of estrogen-withdrawal in monkeys, following a single administration of an estrogen. Diethyl-stilboestrol and ethinyl-estradiol were used.†

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* Aided by a grant from the Rockefeller Foundation.

† The stilboestrol used in this study was diethyl-stilboestrol, for which we are greatly indebted to Dr. J. A. Morrell of E. R. Squibb & Sons. The ethinyl-estradiol was furnished through the courtesy of Mr. Robert Mautner of the Ciba Corporation.

Zuckerman and Palmer,[1] and Bishop, Boycott and Zuckerman[2] have shown that successive injections of stilboestrol cause proliferation of the monkey endometrium, with bleeding as a withdrawal effect.

The present series of experiments was performed on adult female rhesus monkeys which previously had been ovariectomized. Estrogens were administered orally, intramuscularly, and by pellets. Vaginal lavages were made to determine day of uterine bleeding.

Crystalline stilboestrol was administered in sesame oil either orally or intramuscularly. The same material was also made up in the form of 10 mg and 20 mg pellets, which were placed subcutaneously. Ethinyl-estradiol (Ciba) was given orally in the form of 1 mg tablets.

Single doses of stilboestrol varying from 1 mg to 200 mg were given orally (in 5 cc of oil) or intramuscularly (in ½ cc of oil). The time that elapsed before uterine bleeding was noted. There were 23 observations with this method. Stilboestrol was found to elicit typical estrogenic effects in the sex skin. With the larger doses the skin over practically the whole body of the monkey became oedematous. The results are shown in Fig. 1. Three features are particularly noticeable:

1. With the larger doses bleeding was not delayed significantly longer than with the low doses.

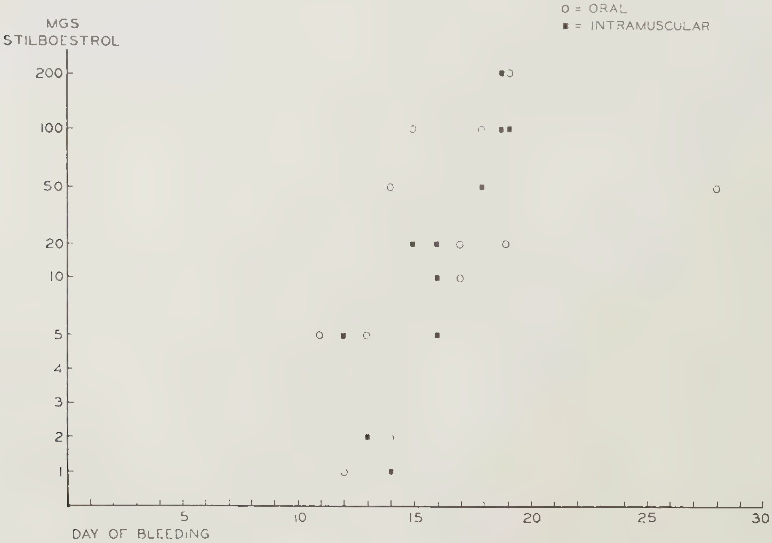

FIG. 1.

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[1] Palmer, A., and Zuckerman, S., *Lancet*, 1939, **1**, 933.

[2] Bishop, P. M. F., Boycott, M., and Zuckerman, S., *Lancet*, 1939, **1**, 5.

2. Oral administration is as effective in prolonging the interval before bleeding as the intramuscular injections.

3. There was no sign of illness or toxicity during or after the treatment.

Pellets containing 10 mg, 20 mg, or 50 mg of stilboestrol were placed subcutaneously in the region of the thigh. One pellet was used in both the 10 mg and 20 mg doses; in the 50 mg doses, two 20 mg and one 10 mg pellets were used. Marked sex skin responses were obtained in every case. The results are shown in Table I. Monkeys 561 and 588 did not bleed, probably due to the gradual involution with the decrease in estrogen The length of time that the sex skin response was maintained demonstrates the continuance of estrogenic action for periods up to 60 or 70 days.

TABLE I.

Ovariectomized *Macacus rhesus* Monkeys Treated with Stilboestrol. Pellets Were Placed Subcutaneously in Thigh.

Monkey	Date of treatment	Wt, g	Stilboestrol in pellets subcutaneously	Uterine bleeding Days after treatment
573	6-12-39	5750	10 mg	55
481	5-31	5200	20 mg	21
561	6-12	3900	20 mg	No bleeding after 85 days. Tremendous sex skin response, fading day 70.
588	5-31	4000	2-20 mg + 1-10 mg	No bleeding after 95 days. Tremendous sex skin response, fading day 85.
520	6-12	4300	2-20 mg + 1-10 mg	Died (TB) on day 65—no bleeding—good sex skin response, fading day 60

An explanation for the early bleeding in monkey 481, as compared with the others in this series, is not available. As far as could be determined, the pellet was not broken or disturbed.

Ethinyl-estradiol[3, 4] in tablet form of 1 mg and 2 mg was given orally in one treatment to 3 monkeys and in 2 treatments to one animal (Table II). It is apparent that 1 mg of this material exerts as prolonged an action, as judged by time elapsing before uterine bleeding, as any dose of stilboestrol given orally or intramuscularly, and that a single treatment is as effective as 2 separate treatments.

Summary. Adult ovariectomized *Macacus rhesus* monkeys were treated with stilboestrol and ethinyl-estradiol.

A. After single oral or intramuscular doses of stilboestrol, vary-

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[3] Inhoffen, H. H., and Hohlweg, H., *Die Naturwissenschaften*, 1938, **6**, 96.

[4] Clauberg, C., and Üstün, *Zentr. f. Gynäk.*, 1938, **62**, 1745.

TABLE II.
Ovariectomized *Macacus rhesus* Monkeys Treated with Ethinyl-estradiol Tablets (Ciba). Each Tablet Contains 1 mg.

Monkey	Date of treatment	Wt, g	Ethinyl-estradiol tablet, orally	Uterine bleeding Days after treatment
574	5-18-39	3700	1 mg	18
574	6-12	3700	1 mg	20
589	6-12	3730	2 mg	19
574	4-24, 4-28	3700	1 mg on 2 days	18 after first day

ing from 1 mg to 200 mg, uterine bleeding occurred in from 11 to 28 days.

B. Oral treatments of stilboestrol appear to exert as prolonged an estrogenic action, as judged by time elapsing before uterine bleeding, as intramuscular injections.

C. Small doses (5-10 mg) of stilboestrol appear to be as effective as large doses (100-200 mg).

D. After oral treatments of ½ mg of ethinyl-estradiol uterine bleeding occurred on days 18-20.

E. No toxic or other untoward effects could be observed, even in heavy doses.

10882

pH of Secretion in Normal Conjunctival Sac Determined by Glass Electrode.

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Adequate quantities of secretion for pH analyses can be collected from the conjunctival sac with little discomfort to the subjects. However, there are 2 components, the viscid corneo-conjunctival film and the watery secretion of the lacrimal gland. Because of the differences in physical properties, a homogeneous solution is not formed and clinical methods of collection do not insure *in vivo* proportions of the 2 components. Therefore, an *in situ* method of determination is preferable. The use of glass electrodes placed in the conjunctival sac to determine the pH of the secretions *in situ* is reported for the first time.

In this study determinations were made of the pH of the conjunctival surfaces of 88 normal adults. A blunt, cylindrical, "L"-shaped electrode was designed to fit into the lower fornix of the conjunctival sac. This electrode was made by Dr. A. E. Cameron* and was used in conjunction with the Cameron pH electrometer. The manufacturer's claim for this apparatus is accuracy to a change in pH of 0.02; however, the *in vivo* readings probably were accurate only to 0.1. The saturated aqueous solution of potassium chloride used to complete the circuit was found to cause conjunctival irritation, but the quantity and duration of contact of this irritant with the conjunctiva were minimized by using an agar bridge impregnated with the solution. The use of a local anesthetic was not necessary and was found to interfere with the results. Failure to completely cover the electrode by conjunctival secretions and contact of the electrode or agar bridge with the skin of the lids were found to result in errors on the acid side. The stimulation of lacrimation by introduction of the electrode into the conjunctival sac resulted in a gradual shift of the reading toward the alkaline side (Table I).

TABLE I.
Effect of Lacrimation on pH of Conjunctiva. Determinations Made at 2-minute Intervals.

Exp.	Subject No.	1	2	3	4	5	6	7	8	9	10	Avg
1.	Initial pH	7.42	7.30	7.30	7.40	7.36	7.17	7.43	7.20	7.24	7.38	7.32
2.	During lacrimation	7.93	7.50	7.60	7.43	7.42	7.49	7.48	7.40	7.32	7.48	7.50
3.	Continued lacrimation	7.92	7.75	7.93	7.61	7.54	7.52	7.61	7.62	7.46	7.68	7.66

However, by minimizing trauma to the lids and conjunctiva and avoiding contact with the cornea, a stable determination could be made on the first eye before the onset of evident tearing. The determinations on the opposite eyes were made after one to 3 minutes to coincide with the period of tearing. In 82 of 88 experimental subjects, an increased alkalinity was found in the second eye, in 6 subjects there was no change. The mean pH for the conjunctival secretion of the eyes of 88 normal adults before lacrimation was 7.23 ± .013 but during lacrimation the mean pH was 7.44 ± .021. On 2 of the authors, 15 determinations were made over a period of 8 months without evidence of injury to the eyes, even though a transitory hyperemia of the conjunctiva followed each determination.

Summary. The pH of the conjunctival surfaces can be determined by placing a specially designed glass electrode in direct contact with the conjunctiva. The method is adaptable to clinical use. A study on 88 normal adults is reported. The results indicate that stimulation of lacrimation results in increased alkalinity of the conjunctival surfaces. A larger series must be studied to establish normal values of pH prior to the onset of lacrimation for it is possible that, with further experience and speed of manipulation, the initial pH determination will show a smaller range and a more nearly neutral reaction.

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10883

Fertility Study of Fresh Eggs by Radio Frequency Conductivity and Dielectric Effect.

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Observations by Romanoff and Grover[1] indicate that as measured by an audio frequency bridge the electrical conductivity of yolk and albumen of fertile eggs decreased while of infertile eggs increased from the beginning of incubation. This suggested the possibility of observations on the whole egg showing differences between fertile and infertile eggs if a radio frequency circuit were used and effects due to eddy current losses observed.

Also work by Romanoff and Sullivan[2] on the refractive index of egg albumen show some differences in fertile from infertile eggs in early stages of incubation. This difference might be observed as an effect due to change in dielectric constant and more easily measured at radio frequency.

Fertilization occurs at least 21 hours before the egg is laid. During this time the development, initiated by fertilization, presumably has stimulated some enzymatic activity and set up chemical changes within the egg.[3] Therefore, there may be some measurable physiochemical difference in fresh fertile and infertile eggs, though this never has been demonstrated.

Apparatus and methods. A radio frequency circuit consisting of a variable condenser, inductance coil and thermoammeter (Fig. 1A), was driven by a link-coupled stable 5-watt generator at frequencies ranging from the 14.0 to 14.4 megacycles.

The method followed was to observe the effect on maximum current and resonant frequency in the radio circuit by introducing an

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[1] Romanoff, A. L., and Grover, H. J., *J. Cell. and Comp. Physiol.*, 1936, **7**, 425.

[2] Romanoff, A. L., and Sullivan, R. A., *Ind. and Eng. Chem.*, 1937, **29**, 117.

[3] Needham, J., *Chemical Embryology*, 1931, p. 247.

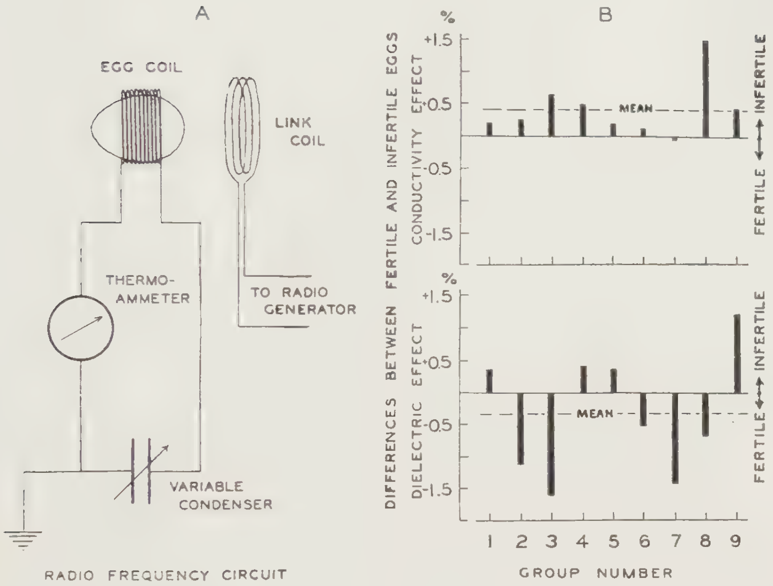

FIG. 1.

A. Circuit diagram: egg coil—length 25 mm, diameter 44 mm, 10 turns, No. 12 copper wire; thermoammeter, 1.5 amp. range; and variable condenser, $30\mu\mu$f.

B. Comparison of differences in 9 groups of fertile and infertile eggs due to the effects of conductivity and dielectric constant, in a radio frequency circuit.

egg into the coil, unlike previous methods in which the material was placed between the condenser plates.[4] These effects depend upon the conductivity of the egg which reduces the current, and upon its dielectric constant which by increasing the coil capacity lowers the resonant frequency. An attempt was made to correlate these effects with the physical characteristics and morphological structure and with the fertility of the whole fresh egg.

The temperature of the room was maintained at 80°F. The eggs on the day of laying were brought to the room and allowed to come to that temperature over night for observation next day.

Experimental. The observed differences of fertile eggs from infertile are shown in Fig. 1B. For comparison there were used 9 groups in pairs, averaging 12 fertile and 8 infertile eggs per group. The eggs were from a flock of White Leghorn hens, and in groups they were selected for narrow ranges of weight and shape factor.

The results indicate that infertile eggs in 8 out of 9 groups showed greater conductivity than fertile eggs. Dielectric constant was found to be higher for fertile than infertile eggs. This was less evident for individual groups but the difference of the mean of the

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[4] Richards, W. T., and Loomis, A. L., *Proc. Nat. Acad. Sci.*, 1929, **15**, 587.

TABLE I.

Relationship Between Energy Absorption and Resonance in Radio Frequency Circuit and Egg Weight, Shape and Proportional Amounts of Contents.

Ammeter reading in amperes	Frequency dial setting in divisions	Width of res. curve at ½ max. in divisions	Egg wt, g	Egg shape $\frac{\text{diam.}}{\text{length}}100$	Albumen %	Yolk %	Shell %	No. of eggs
1.291-1.335	41.7	22.8	61.0	75.5	63.2	28.3	8.6	6
1.336-1.380	45.3	23.1	60.0	75.2	60.9	29.7	9.4	26
1.381-1.425	49.4	23.8	58.1	74.5	59.4	31.1	9.7	39
1.426-1.470	52.1	24.9	55.8	73.9	59.1	30.9	9.9	51
1.471-1.515	54.6	25.8	54.0	72.1	58.5	31.5	10.0	26

Based on 148 infertile eggs.

TABLE II.

Influence of Replaced Egg-contents on Energy Absorption and Resonance in Radio Frequency Circuit.

Observations	Whole egg	Eggshell empty	Eggshell filled with distilled water	Eggshell filled with albumen	Eggshell filled with yolk
Conductivity:					
Max. ammeter reading with egg*	0.938	1.470	1.462	0.913	1.090
Per cent current reduction	37.5	3.0	3.1	40.0	28.0
Dielectric effect:					
Resonance frequency dial reading with egg†	32.8	75.3	35.2	32.6	32.4
Per cent frequency shift	1.49	0.17	1.42	1.50	1.50

* Maximum ammeter reading without egg 1.515.

† Resonance frequency dial reading without egg 81.0.

The average weight of whole eggs 59.8 g.

total number of infertile eggs and of the mean of the total number of fertile eggs was almost twice the probable error.

These changes were greater with the increase in egg weight (Table I). The egg shape, defined as the ratio of diameter to length, produced similar effects. The influence of egg weight was also modified by changes of proportional amounts of contents. Relative to the effect of different separations between the upper part of the coil and the egg it was found that the meter readings and frequency dial settings vary only slightly if the variations in weight and shape factor (d/1) of the egg are taken into consideration. The correction was made by multiplying each reading by a factor which is:

$$\mathrm{Wt.}\ x(d/1) \div \mathrm{Wt.}_{av}x(d/1)_{av}.$$

Table II presents evidence that most of the current reduction is due to the albumen content of the egg. As for the dielectric effect it is seen that both albumen and yolk had almost the same values as had the eggshell filled with distilled water.

Summary. The experiment introduces a new method of measuring conductivity and dielectric effects, independent of electrodes in contact with the material, and is especially adaptable for observations of these properties of a whole egg.

The results indicate that conductivity is lower and dielectric constant higher in fertile fresh eggs than in infertile.

10884

The Egg-count Index of *Trichocephalus vulpis* Infections in Dogs.*

Max J. Miller. (Introduced by E. C. Faust.)

From the Department of Tropical Medicine, Tulane University of Louisiana, New Orleans, La.

Leuckart[1] first attempted to determine the egg output of the female *Trichocephalus trichiurus,* the whipworm of man. He calculated that the uterus contained 58,000 eggs, and that 4 to 6 times this number were passed each year, making the daily egg output approximately 1000. From the number of eggs passed daily in the feces of a patient, and from the number of worms found to be harbored by

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* Aided by a grant from E. Merck and Co.

[1] Leuckart, R., *Die Menschlichen Parasiten*, 1876, 2nd ed., 188 pp.

the patient when brought to autopsy shortly thereafter, Moosbrugger[2] estimated that each female laid 3333 eggs daily. On the other hand, Manalang,[3] working with colonic feces of autopsied persons, arrived at a figure of 310 eggs per gram of formed feces, which, on the basis of an average daily fecal output of 150 g, would amount to a daily egg output of about 46,000. Correa and Mellone,[4] utilizing the same type of material as did Manalang, calculated that each female *T. trichiurus* laid 315 eggs per gram of formed feces. Since the results obtained by Leuckart and Moosbrugger differ widely from those of the later workers, and since the number of intestinal helminth eggs passed in feces is the only accurate index to the worm burden harbored by the host, it is important to know which of the two groups of results is the more accurate. In order to gain some information on this question, a study was made on the egg output of *T. vulpis,* the whipworm of dogs, a species closely related to that of man.

Six whipworm-free dogs, 2 pups and 4 adults, were utilized. The dogs all harbored hookworms when first examined but were freed of this infection prior to their experimental inoculation with embryonated whipworm eggs. As soon as each animal became positive, egg counts were carried out according to the method of Stoll,[5] and the daily egg output was determined by making counts on a composite sample of feces collected for 3 consecutive days. After the egg output showed a constancy over a period of at least 18 days, the animals were sacrificed, the worms removed, separated as to sex, and counted.

The results of the experiment are presented in Table I. The 6 animals were found to harbor a total of 1731 worms—1148 females

TABLE I.

Egg Output of *Trichocephalus vulpis* as Determined by Ante-mortem Egg Counts, and Post-mortem Worm Counts.

Dog No.	C1	C2	C3	C4	C5	C6	Total
Daily ante-mortem egg output	107,000	368,000	125,000	96,000	1,152,000	487,000	2,335,000
Females found	52	271	26	25	558	216	1,148
Males ”	7	171	11	10	287	97	583
Total	59	442	37	35	845	313	1,731
Avg No. of eggs per female	2,058	1,358	4,808	3,840	2,065	2,255	Avg total 2,035
Avg No. of eggs per worm	1,814	833	3,379	2,743	1,363	1,558	Avg total 1,349

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[2] Moosbrugger, *Med. Corr. Blatt des Wurttemberg. artz Landesver.*, **61**, 277.

[3] Manalang, C., *Philipp. J. Sci.*, 1928, **35**, 11.

[4] Correa, M., and Mellone, O., *A Fohla Medica*, 1938, April.

[5] Stoll, N., and Hausheer, W., *Am. J. Hyg.*, 1926, **6**, 134.

and 583 males. The total average number of eggs passed daily was found to be 2,335,000. Dividing this figure by the total number of females recovered, it is found that each female *Trichocephalus vulpis* lays a daily average of 2035 eggs. Dividing the total daily egg output by the total number of worms recovered, it is found that each 1350 eggs in the feces represents one worm in the host. The average daily egg output in the different animals varied considerably (*i. e.*, from 833 to 3379). The lighter infections showed a larger daily egg output per worm.

The ratio of male to female whipworms harbored by the dogs was found to be 1 :2. However, a compilation of the number of male and female *Trichocephalus trichiurus* found at autopsy by various workers gives a ratio of 1 : 1.2.

Conclusions. While the results of the present study on the egg output of *Trichocephalus vulpis* cannot be directly applied to that of *T. trichiurus*, they may provide some idea of the true output of the latter. If comparison is permitted with the results obtained by previous workers on *T. trichiurus*, it may be concluded that Leuckart's and Moosbrugger's calculations are much closer to the true ones than are those of Manalang and Correa and Mellone.

10885

Studies on Embryonation and Hatching of the Eggs of the Dog Whipworm, *Trichocephalus vulpis*.*

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The embryonation of *Trichocephalus vulpis* eggs was studied after mechanical agitation by centrifugalization, immersion in brine for a short period, and in sodium chloride solutions of different strengths. The extra-corporeal hatching of fully-embryonated *T. vulpis* eggs was also studied.

The eggs were collected from the feces of infected dogs by sieving, alternate sedimentation and decantation, and finally, in all experiments except the first, by brine centrifugal flotation. All embryonation experiments were carried out at temperatures of 33° to 36°C.

In the experiment to determine the effects of centrifugalization and

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* Aided by a grant from E. Merck and Co.

immersion in brine on the embryonation of *Trichocephalus vulpis* eggs, 4 types of cultures were studied. The eggs in the first type were collected by sedimentation alone and served as control; in the second, by sedimentation followed by centrifugalization at 2600 r.p.m. for 2 minutes; in the third, by sedimentation followed by immersion in brine for 5 minutes; in the fourth, by sedimentation and brine centrifugal flotation. In the experiment to determine the effects of sodium chloride on the embryonation of *T. vulpis* eggs, saline solutions from 1/1000 N to 3 N were used. Eggs in distilled water served as control.

The results showed that almost 100% of the eggs subjected to centrifugalization and immersion in brine were completely developed after 13 and 14 days respectively, and eggs subjected to both, after 9 days of incubation. Only 15% of the control eggs were fully embryonated after 19 days of incubation. Eggs cultured in sodium chloride solutions of 1/1000 to 1/10 N completed their development in 12 days, while control eggs in distilled water required 23 days. Eggs cultured in a 5/10 N solution required 16 days to complete their development, and a small percentage showed a partial collapse of the inner layer of the egg shell, although the larvae within appeared quite viable. About 40% of the eggs cultured in normal salt solution contained vermiform larvae after 12 days of incubation. However, there was no further development, and after 19 days all eggs appeared to be degenerate. There was no development of eggs in a 3 N solution, and all eggs were dead within 5 days.

The extra-corporeal hatching of *Trichocephalus vulpis* eggs was studied at a temperature of 37°C in artificial gastric juice (0.8% pepsin in an 0.5% solution of HCl) and artificial pancreatic juice (1.5% solution of pancreatin in distilled water brought to a pH of 7.3 with Na_2CO_3).

The eggs did not hatch in gastric juice, nor did hatching occur in distilled water. Up to 2% hatched in pancreatic juice alone, but never a larger percentage. Eggs placed in pancreatic juice following a previous immersion in gastric juice showed a much higher percentage of hatching. Thus, 10% of eggs previously placed in gastric juice for 30 minutes, were hatched after 19 hours in pancreatic juice, and from 25% to 50% of eggs, previously placed in gastric juice for 20 hours, were hatched after 2 hours in pancreatic juice. *In vivo* studies on hatching confirmed the *in vitro* findings. Hatching was never found to occur in the stomach of dogs, but always in the small bowel. However, hatching occurred more rapidly in the experimental animal's digestive tract. Eggs introduced by stomach tube were found hatching within 30 minutes.

Summary and Conclusions. (1) Since *Trichocephalus vulpis* eggs are stimulated in their development by previous subjection to mechanical agitation by centrifugalization, and to immersion in brine, the results of studies on these, and probably other nematode eggs subjected to these influences are not applicable to eggs developing in nature. (2) Sodium chloride in low dilutions (1/1000 N to 1/10 N solutions) stimulates the embryonation of *T. vulpis* eggs. Higher concentrations (1 to 3 N solutions) inhibit development, collapse the inner layer of the egg shell, and finally kill the embryo. It is not unlikely that other salts present in the soil in small concentrations may also influence the embryonation of these eggs. Thus, the salt content of soils may conceivably play an important rôle in the epidemiology of whipworm disease. (3) From 25% to 50% of *T. vulpis* eggs can be hatched by subjecting them in turn to artificial gastric juice for 20 hours and artificial pancreatic juice for 2 hours. The fact that eggs must remain in the gastric juice for a period of time before they will hatch in pancreatic juice suggests that the rapidity with which eggs pass through the stomach of the host may influence the rate of hatching, and thus the resulting intensity of infection in the host.

10886

Sulfanilamide and Sulfapyridine in Treatment of Experimental *B. Friedländer* (*Klebsiella pneumoniae*) Infections of Mice.

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Buttle, Parish, McLeod and Stephenson[1] found sulfanilamide in dose of 0.025 g by oral administration twice daily practically ineffective in the treatment of mice inoculated intraäbdominally with varying amounts of 18-hour broth culture of *B. friedländer;* of 70 treated mice only 12 survived the minimal amount of culture for 12 days, whereas 4 out of 40 untreated controls remained alive. All treated and control mice inoculated with larger amounts of culture perished so that the compound was found to give only temporary protection. Bliss, Feinstone, Garrett and Long,[2] using type B of

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[1] Buttle, G. A. H., Parish, H. J., McLeod, M., and Stephenson, D., *Lancet*, 1937, **1**, 681.

[2] Bliss, E. A., Feinstone, W. H., Garrett, A. W., and Long, P. H., PROC. SOC. EXP. BIOL. AND MED., 1939, **40**, 619.

B. friedländer observed essentially similar results in that none of 45 treated mice survived when inoculated with sufficient culture to cause fatal infections of 30 controls.

Our experiments were conducted with a strain freshly cultivated from the cerebrospinal fluid of a fatal case of *B. friedländer* meningitis secondary to a chronic ethmoiditis* due to the same organism. Its virulence was determined by giving mice 0.01, 0.02, 0.03, 0.04, 0.05, 0.08, and 0.1 cc of 24-hour broth culture by intraäbdominal inoculation, 2 mice being used for each dose. The 2 mice inoculated with 0.01 cc survived; both inoculated with 0.02 cc died in 5 to 6 days while the remaining mice succumbed in 1 to 4 days, all with positive heart blood cultures. In the treatment-experiments mice were inoculated with 0.05 cc of 24-hour broth culture corresponding to between 2 and 3 minimal lethal doses. All of 12 untreated controls succumbed in 24 to 72 hours with positive heart-blood cultures.

Since difficulty was experienced in giving mice accurate doses of the compounds by mouth, they were administered in solution by subcutaneous or intraäbdominal injection. For this purpose 0.4 g of sulfanilamide was dissolved in 50 cc hot saline giving 0.008 g per cc; 0.2 g sulfapyridine was dissolved in 200 cc of hot saline solution giving 0.001 g per cc.

In one experiment 16 mice were given 0.160 g sulfanilamide per kilo by *subcutaneous* injection immediately after intraäbdominal inoculation with 0.05 cc of 24-hour broth culture followed by a second dose of the drug 6 hours thereafter and repeated twice daily (10 A.M. and 3 P.M.) for 5 days. Four additional mice, which were infected but not treated, served as controls. They all succumbed in 24 to 48 hours after inoculation. Of the 16 treated animals 3 survived for 1 to 2 or 3 days beyond the untreated controls, indicating that sulfanilamide in the dose administered probably showed some slight protective effect.

In the second experiment 20 mice were inoculated with 0.05 cc of 24-hour broth culture; 4 kept as untreated controls died in 24 to 72 hours. Sixteen of the mice were given 0.160 g *sulfanilamide* by *intraäbdominal* injection immediately after inoculation, 6 hours thereafter and then twice daily (10 A.M. and 3 P.M.) for 5 days. Of the 16 treated mice 2 survived while the lives of 6 were prolonged for 3 to 6 days beyond the untreated controls, indicating some therapeutic effect probably because of more rapid absorption than occurred after subcutaneous injection.

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* Patient a male; age 36 years; weight 154 lbs; sulfapyridine administered orally in dose of 1.0 g every 4 hours beginning about 3 days after onset of meningitis and continued for 8 days when death occurred (necropsy denied). Marked clinical and spinal fluid improvement during first 4 days of treatment.

In a third experiment 20 mice were inoculated with 0.05 cc of 24-hour broth cultures; 4 kept as untreated controls died in 24 to 72 hours. Sixteen of the mice were given 0.160 g *sulfapyridine* by *intraäbdominal* injection immediately after inoculation with a second dose 6 hours later and thereafter twice daily (10 A.M. and 3 P.M.) for 5 days. Of these, 4 survived for 12 days when the experiment was terminated while the lives of 6 were prolonged 1 to 5 days beyond the untreated controls.

Summary. Sulfanilamide by subcutaneous injection in dose of 0.160 g per kilo slightly prolonged the lives of 3 out of 16 mice when given immediately, 6 hours later and thereafter twice daily for 5 days after intraäbdominal inoculation with *B. friedländer* in a dose fatal in 24 to 72 hours. When the compound in the same dose was given by intraäbdominal injection in the same manner to 16 mice, 2 survived 12 days when the experiment was terminated, while the lives of 6 were prolonged for 3 to 6 days beyond the untreated controls. Of the total of 32 treated mice, 2 survived and the lives of 9 were prolonged, whereas all of 8 untreated controls succumbed in 1 to 3 days after inoculation.

Sulfapyridine was somewhat more effective. Of 16 mice given 0.160 g by intraäbdominal injection immediately after inoculation, 6 hours later and thereafter twice daily for 5 days, 4 survived while the lives of 6 were prolonged 1 to 5 days beyond the survival of 4 untreated controls which succumbed in 24 to 72 hours after inoculation. All treated and untreated mice succumbing gave positive heart-blood cultures. All drug controls given both sulfanilamide and sulfapyridine by subcutaneous and intraäbdominal injection survived the period of 12 days, when the experiments were terminated.

10887

Sulfanilamide and Sulfapyridine in Treatment of Experimental *B. coli* (*Escherichia coli*) Infections of Mice.

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Clinically sulfanilamide has proven useful in the treatment of some cases of urinary-tract infections due to *B. coli* as well as in some cases of septicemia due to this organism. It is possible, however, that not all strains are equally susceptible to the effects of this compound *in vitro* and *in vivo* and for this reason an *in vitro* test for bacteriostatic and bactericidal effects of sulfanilamide upon strains of *B. coli* isolated from the urine of urinary-tract infections, may be helpful in determining the dosage to employ in treatment.

In experimental *B. coli* infections of mice produced by intraäbdominal inoculation Cooper, Gross, and Lewis[1] found sulfanilamide effective, 55% of 40 treated mice surviving inoculation with fatal amounts of culture. They also observed that sulfapyridine was somewhat more effective since 80% of a group of 10 mice survived.

The strain employed in our experiments was cultivated in pure culture from the urine of a case of pyelonephritis of 5 years' duration occurring in a boy 17 years of age. A concentration of about 0.5% sulfanilamide in 5 cc of broth seeded with approximately 200,000 bacilli was required for bacteriostatic effects while a concentration as high as 1% was not completely bactericidal, indicating that this particular strain was above the average in resistance to sulfanilamide. Its virulence for mice was determined by giving 0.02, 0.05, 0.1, 0.2, 0.3, and 0.4 cc of 24-hour broth culture intraädominally, 2 mice being used for each dose. Amounts of 0.2, 0.3 and 0.4 cc proved fatal in 1 to 3 days with positive heart-blood cultures while mice inoculated with the smaller doses survived. For the therapeutic tests mice were inoculated intraäbdominally with 0.3 cc of 24-hour broth culture, corresponding to between 1 and 2 minimal lethal doses, which proved fatal for all untreated controls in 24 to 48 hours after inoculation.

Since difficulty was experienced in giving mice accurate doses of the compounds by mouth, sulfanilamide was administered in solution by subcutaneous and intraäbdominal injection. For this purpose 0.4 g was dissolved in 50 cc of hot saline giving 0.008 g per cc. Sulfapyridine was administered by intraäbdominal injection; for this purpose 0.2 g was dissolved in 200 cc of hot saline, giving 0.001 g per cc.

In one experiment 10 mice were given 0.160 g per kilo of *sulfanilamide* by *subcutaneous* injection 2 hours after intraäbdominal inoculation with 0.3 cc of 24-hour broth culture followed by a second dose 4 hours later and thereafter by 2 doses daily (10 A.M. and 3 P.M.) for 5 days. None of the mice survived although the lives of 3 were prolonged for 1 to 3 days beyond those of 4 untreated controls, all of which succumbed in 1 to 2 days after inoculation. The unfavorable results in treatment were probably due to the unusually high resistance of the strain to sulfanilamide as indicated by the results of the *in vitro* tests.

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[1] Cooper, F. B., Gross, P., and Lewis, M., PROC. SOC. EXP. BIOL. AND MED., 1939, **40**, 34.

Essentially similar results were observed in a second experiment in which 10 mice were given 0.160 g of *sulfanilamide* per kilo by *intraabdominal* injection 2 hours after inoculation with 0.3 cc of 24-hour broth culture, followed by a second dose 4 hours later and thereafter by 2 doses daily (10 A.M. and 3 P.M.) for 5 days. None of the mice survived, although the lives of 3 were prolonged 2 to 3 days beyond those of 4 untreated controls, all of which succumbed in 1 to 2 days after inoculation.

Treatment with *sulfapyridine* did not yield any better results with this strain. Of 10 mice given 0.160 g per kilo by *intraäbdominal* injection 2 hours after inoculation with 0.3 cc of 24-hour broth culture, followed by a second dose 4 hours later and thereafter twice daily (10 A.M. and 3 P.M.), for 5 days, none survived although the lives of 4 were prolonged by 1 to 3 days beyond the survival of 4 untreated controls, all of which succumbed in 1 to 2 days after inoculation. In each experiment all treated and untreated mice succumbing gave positive heart blood cultures; two treatment controls survived 12 days, when the experiment was terminated.

Summary. Sulfanilamide by subcutaneous and intraäbdominal injection and sulfapyridine by intraäbdominal injection in dose of 0.160 g per kilo slightly prolonged the lives of some mice when given 2 hours after intraäbdominal inoculation with *B. coli* in a dose fatal in 24 to 48 hours, followed by a second dose 4 hours later and thereafter twice daily (10 A.M. and 3 P.M.) for 5 days. All of the treated mice succumbed with positive heart-blood cultures and the unfavorable results are believed to be due to the fact that the strain of *B. coli* employed was one unusually resistant to the bacteriostatic and bactericidal effects of sulfanilamide as indicated by the results of *in vitro* tests. All treatment-controls lived for 12 days, when the experiments were terminated.

10888 P

Intersexuality in Adult Brown Leghorn Male as a Result of Estrogenic Treatment During Early Embryonic Life.*

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Since the publication of Lillie's[1] work on the free-martin many investigators have attempted to duplicate this condition in birds by transplanting gonads of either sex onto the chorio-allantoic membrane. While these early attempts were probably invariably negative it is now known that early application of the chemically prepared sex hormones will cause varying modifications in development of the embryonic reproductive system of birds as well as other forms.[2-8]

The present study records post-embryonic transformations in embryos that had been treated with estrogens prior to the period of sexual differentiation. Brown Leghorn eggs received single injections between the 3rd and 5th days of incubation and the treated embryos were allowed to hatch and mature. The estrogens used were progynon-B (1500 to 3000 R.U.) and theelin (0.5 to 1 mg). The quantity administered per injection was .05 or 0.5 cc. A total of 410 eggs were treated in this set of experiments of which 69 hatched. Of this number 51 (24♂ and 27♀) developed beyond the period of sexual differentiation and form the basis of this study.

The males of this experiment reveal an interesting sexual transformation. The sexes could invariably be distinguished at the period

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* This investigation has been aided by a grant from the Rockefeller Foundation to the University of Chicago. Grateful acknowledgments are made to Dr. Erwin Schwenk, Schering Corporation, for progynon-B; to Dr. Oliver Kamm, Parke Davis & Co., and Prof. F. C. Koch, the University of Chicago, for theelin; to Mrs. Sally Johnson, A. M. Guhl and M. Posner for excellent assistance.

[1] Lillie, Frank R., *J. Exp. Zool.*, 1917, **23**, 371.

[2] Kozelka, A. W., and Gallagher, T. F., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 1143.

[3] Willier, B. H., Gallagher, T. F., and Koch, F. C., *Proc. Nat. Acad. Sci.*, 1935, **21**, 625; *Physiol. Zool.*, 1937, **10**, 101.

[4] Wolff, E., and Ginglinger, A., *Arch. d'Anat., d'Hist., et d'Emb.*, 1935, **20**, 219.

[5] Dantchakoff, V., *Bull. Biol.*, 1936, **70**, 241; 1937, **71**, 269; 1938, **72**, 187.

[6] Domm, L. V., and Dennis, E. A., PROC. SOC. EXP. BIOL. AND MED., 1937, **36**, 766.

[7] Greene, R. R., Burrill, M. W., and Ivy, A. C., *Sci.*, 1937, **86**, 200; 1938, **87**, 396; 1938, **88**, 130.

[8] Moore, C. R., PROC. SOC. EXP. BIOL. AND MED., 1939, **40**, 544.

of sexual differentiation. Some males appeared quite normal at this time while in others feminizing effects were evident in the plumage, but in none of these cases did a complete henny plumage appear before the end of the first year though this has occurred in recent experiments. However, following the first molt many of them developed a henny plumage while others developed the plumage prevalent preceding the molt.† Head furnishings were invariably masculine though in some they became strikingly feminine during the molting period and remained so for a time, then again became masculine. Most of them are known to crow and tread though sexual libido does not appear to be as intense as in normals.

Post-mortem revealed striking modifications of the reproductive system. The left testis invariably showed a remarkable change in size and form, being smaller than the normal and instead of having the characteristic ellipsoid shape it is invariably flattened and ovary-like. The smooth even surface characteristic of the normal testis is replaced by an irregular uneven surface similar to that of the ovary. The right testis is rarely so strikingly modified, being larger, and its modifications are of the same character though less extreme.

Variously developed oviducts occur, though in some they are entirely absent. The left oviduct is generally better developed than the right and in certain cases appears entirely female. In a few instances only anterior parts of the oviduct persisted. The right, where present, is always better developed than that found in normal hens. In a few cases the right appears to be essentially a normal oviduct. Vasa deferentia are found though they are not always as prominently convoluted nor as large in diameter as those of cocks.

Fourteen cases have been studied histologically. In these the left gonad is an ovotestis with irregular surface cortex underlying which are testis tubules showing varying degrees of spermatogenesis. The cortical layer, composed of a stroma containing cortical cords, varies in depth, and occasionally one finds distended medullary tubules between it and the underlying testis tubules. In one case scattered follicles of varying size, with normally developed ova containing yolk granules and nucleus, have been found in the cortex. Modifications of the right gonad are similar but less extreme, and the gonad, though noticeably modified macroscopically, may not reveal a significant cortical component. None of our cases have revealed follicles in the right gonad.

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† Seven individuals remain under observation and have now about completed the second yearly molt. Four of these have again developed the plumages present during the preceding year, 2 of which were female and 2 male. That of the remaining 3 was principally intermediate in character and fluctuated somewhat during the year. Two of these are developing intermediate plumage while in the other the new plumage is male.

The degree of sex-reversal attained is probably proportional to the quantity of estrone introduced though considerable variation occurs with any given dosage. The basis for the changes observed is the intersexual composition of the zygotically male gonads brought about by administration of estrone. Differences in response of gonads is attributable to differences occurring in normal development of testis rudiments on opposite sides. The left testis passes through a stage from approximately 7 to 11 days during which a definite germinal epithelium containing germ cells is present over the entire free surface, whereas the right only occasionally shows scattered traces of this structure. It is this germinal epithelium of the testis, particularly the left, which becomes activated by the introduced estrones and develops into ovarian cortex. The differences in response between testes clearly indicates that they basically possess the same fundamental asymmetrical condition long known to be characteristic of right and left ovaries in birds.

The phenomenon of sex-inversion in the fowl has come to be associated entirely with a masculinization of the female while the opposite condition, a spontaneous development of female characters by genetically determined males has not been generally admitted. These experiments show that such transformations are possible and it is therefore altogether probable that some of the hermaphroditic fowl reported in the literature may actually have been feminized males, owing to development of ovarian cortex on testes, rather than masculinized females as interpreted.

10889

Electrocardiographic Changes in Ethylene Glycol Poisoning.

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During the course of some experiments on the pathology of ethylene glycol poisoning of pups some striking alterations in cardiac rhythm were noted. These observations prompted the formulation of a series of experiments designed to determine the cause of the cardiac arrhythmia. Repeated electrocardiograms were made on 4 littermate puppies 6 weeks of age and on 4 adult dogs. The ethylene glycol was administered orally as a 4% solution which the animals were permitted to drink *ad libitum.* A record was made of the daily quantity of the solution imbibed by each animal. Two pups and 2 adult dogs drank a solution of c.p. ethylene glycol while the others imbibed water which contained an equivalent amount (4%) of a commercial antifreeze (Prestone) which is largely ethylene glycol. The survival period of pups and adult dogs ranged from 100 to 174 hours and 74 to 136 hours respectively.

Control electrocardiograms were recorded twice on each animal before the administration of ethylene glycol. The heart rate of the pups as shown by these electrocardiograms ranged from 130 to 190 per minute, whereas, that of the adult dogs varied from 85 to 160 per minute. The cardiac rhythm was quite regular.

Following the initiation of the ethylene glycol poisoning the electrocardiograms were recorded daily until toxic symptoms developed, at which time the interval between the successive records was reduced to from one-half to 4 hours. When the electrocardiograms were taken the animals were suspended on either a wooden or a canvas frame. The suspension apparatus maintained the dogs in a standing posture. Needle electrodes introduced into the skin and subcutaneous tissue were used to pick up the heart potentials. Lead I electrocardiograms were recorded from symmetrical regions of the skin overlying the middle and caudal portion of each scapula. These skin areas were marked so that repeated electrocardiograms could be recorded from exactly the same place. Since it was the primary object of these experiments to ascertain the cause of the arrhythmia, it was deemed unnecessary to record more than one lead.

Examinations of the electrocardiograms recorded at various stages during the poisoning episode showed significant changes in the functional activity of the pacemaker. In pups, the characteristic findings displayed by the electrocardiograms may be summarized as follows: (1) A profound bradycardia, the heart rate in most instances being reduced to less than one-half that of the original normal value (normal rate of 130 to 160 reduced 60 to 70 per minute); (2) exaggerated sinus arrhythmia showing marked cyclic changes in the R-R intervals and rhythmic changes in the height of the R waves, the magnitude being greatest when the R-R intervals were the longest (Fig. 1); (3) sino-auricular block showing typical S-A dropped beats; (4) slight displacement of the S-T segments and in some instances diphasic T waves; (5) the P-R and Q-S intervals of all records remained within normal limits.

Electrocardiograms of the poisoned adult dogs differed considerably from those obtained in pups. The sinus arrhythmia was not pronounced. The bradycardia, though quite marked was not as

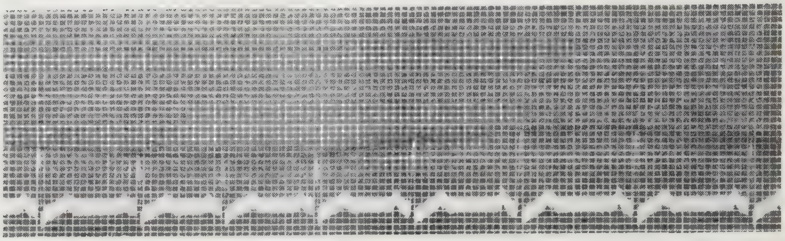

FIG. 1.

Electrocardiogram of pup No. 1 poisoned with 4% c.p. ethylene glycol—1-22-1938. Time 5:15 P.M. Interval of 170¼ hours after the beginning of the poisoning.

Heart rate 75/min.

Record No. 24 showing sinus arrhythmia with cyclic changes in the amplitude of the R waves, the amplitude being greatest when the R-R intervals are the longest.

extensive, the lower limit of 70 to 75 per minute being reduced from the control pulse rates of 85 to 160 per minute. The pronounced slowing of the heart developed shortly before death and was sometimes preceded by a definite tachycardia. One of the adult dogs whose control heart rate was 85 per minute showed only an increase in heart rate, reaching 115 per minute 10 hours before death. One electrocardiogram recorded from one of the dogs 2 hours before death, showed a prolonged P-R interval greater than 0.20 seconds; the electrocardiogram in this instance measured 0.28 seconds. The irregularity of cardiac rhythm was caused in part by S-A dropped beats but also by nodal and ventricular premature contractions. The most constant and striking alteration of the electrocardiograms of adult dogs occurred in the S-T segments and in the T wave (Fig. 2). An S wave of 1.5 to 3 mm followed by a low take-off and a convex curvature of the S-T segment, and a large diphasic T wave were found to be a more or less constant S-T complex. The magnitude

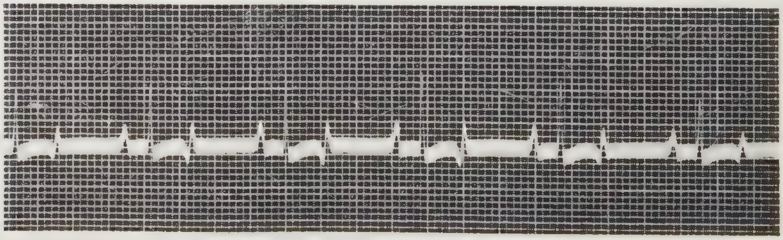

FIG. 2.

Electrocardiogram of adult dog No. 10 poisoned with 4% c.p. ethylene glycol—1-20 1938. Time 10:45 P.M. Interval of 126¾ hours since the start of the ethylene glycol poisoning.

Heart rate 75/min.

Record No. 9 showing the typical S-T changes and diphasic T wave characteristic of ethylene glycol poisoning.

of the diphasic T wave usually measured 2 mm as a negative deflection and 3-5 mm as a positive deflection. In 2 of the adult dogs the positive phase of the diphasic T wave appeared first; in one, the negative phase was the first to appear and in the other dog there was a reversal of the initial deflection, being negative in the first 3 records showing diphasic T waves, positive in the next 4, and again negative in the last 3.

Conclusions. Bradycardia which was sometimes preceded by tachycardia developed in dogs poisoned with ethylene glycol. The cardiac arrhythmia of both immature and mature dogs were both regular and irregular in character. The regular alteration of rhythm was caused by an exaggerated sinus arrhythmia. The irregularity of cardiac rhythm of pups was caused by sino-auricular block, whereas, in adult dogs, it was caused not only by sino-auricular block but also by nodal and ventricular extra-systoles. A partial A-V block developed in only one of the 4 adult dogs and in this instance it did not occur until 2 hours before death. Atrio-ventricular block, therefore, appeared to be an exception rather than a rule in ethylene glycol poisoning. The deep S waves and the displacement and curvature of the S-T segment combined with a diphasic T wave were constant findings in the electrocardiograms of adult dogs after the developmeent of toxic symptoms of ethylene glycol poisoning. Similar S-T changes occurred in pups but much less extensively. The abnormal S-T complex (deep S wave, low take-off and convex curvature of S-T segment followed by a diphasic T wave) therefore appeared to be a characteristic alteration of the electrocardiograms of dogs poisoned with ethylene glycol. The electrocardiograms of dogs poisoned with "Prestone" were identical with those poisoned with c.p. ethylene glycol.

10890

Effects of Morphine Sulphate on Hypothalamus of the Cat.

Jules H. Masserman. (Introduced by E. M. K. Geiling.)

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In 16 animals under light ether anesthesia, a needle electrode was inserted stereotaxically into the hypothalamus and observations were made as to the changes in the responses of this structure to faradic stimulation after the intra-hypothalamic or the intraperitoneal injection of a solution of morphine sulphate. Corresponding data were also obtained in 8 unanesthetized cats previously prepared under nembutal anesthesia by the insertion of separate needle electrodes into the hypothalamus and the opposite posterior cruciate gyrus.[1] The results of these experiments may be summarized as follows:

Acute Preparations. Stimulation of the anterior hypothalamus with from 2 to 8 volts of a 60 cycle current produced various degrees of piloerection, cyclodilatation with retraction of the nictitating membranes, salivation, forced urination, extrusion and fanning of the claws, champing and licking of the jaws, lashing of the tail, running and pawing movements of the fore-legs, and biphasic changes in the respiration and blood pressure. The injection directly into the hypothalamus of from 0.05 to 0.10 cc of Locke's solution containing from 1 to 10 mg of morphine sulphate or the intraperitoneal administration of from 30 to 180 mg of the drug produced no discernible effects in the anesthetized animal other than a slight myosis and a variable decrease in the rate of respiration; moreover, the responses of the hypothalamus to subsequent faradic stimulation were not consistently affected either in nature or degree.

Recovery Preparations. Electrical stimulation of the hypothalamus in an unanesthetized animal produced the same vegetative effects as in the acute preparation, and in addition, coördinated vocal and muscular activity suggestive of anxiety, fear and rage.[1] In contrast, stimulation of the cruciate cortex with weak currents (2 to 4 volts A.C.) produced only maintained flexion or extension of the legs, torsion of the body and occasionally slight piloerection or cyclodilatation, whereas stronger currents (4 to 12 volts) induced greater vegetative responses and muscular convulsions which progressed from the contralateral leg to involve the entire body. The intra-hypothalamic or intra-cortical injection of from 1 to 10 mg of morphine sulphate in 0.1 cc of Locke's solution again produced no evident effects, but the injection of from 30 to 180 mg of the drug intraperitoneally caused variable myosis, motor restlessness (3 of 8 animals) and finally, a stupor or coma which persisted for from 8 to 30 hours. However, the local or the intraperitoneal injection of morphine sulphate produced no consistent changes in the characteristic responses to electrical stimulation of either the hypothalamus or the cruciate cortex, other than that in the former case the animals which received more than 60 mg of morphine sulphate would cease their pseudo-affective behavior and relapse into stupor immediately after the cessation of the hypothalamic stimulus.

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[1] Detailed descriptions of the experimental methods employed in these studies and observations that were used as controls in the present work are included in previous reports from this laboratory (Masserman, J. H., *Arch. Neurol. and Psychiat.*, 1937, **37**, 617; *ibid.*, 1938, **39**, 1250; *ibid.*, 1939, **41**, 504; Masserman, J. H., and Haertig, E. W., *J. Neurophysiol.*, 1938, **1**, 350; Masserman, J. H., *J. Pharm. and Exp. Ther.*, 1938, **64**, 335). Thanks are due to Mr. John Austin for technical aid in the present study.

Conclusion. In the cat, the local injection of from 1 to 10 mg of morphine sulphate in Locke's solution or the intraperitoneal administration of from 30 to 100 mg of the drug does not affect the reactivity of either the cruciate cortex or the hypothalamus to faradic stimulation, except that in animals which receive more than 60 mg intraperitoneally the hypothalamic responses are limited to the duration of the stimulus.

10891

Effect of Sulfanilamide on the Isolated Frog Heart.

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A study was made of the effects of sulfanilamide on the isolated frog heart using the Straub-Fuehner method. The solutions of sulfanilamide used ranged from 0.05% to 0.50% and were made up in frog heart Ringer's. This solution was used between sulfanilamide perfusions to return the heart to normal.

All concentrations caused an increase in amplitude, more marked in the higher concentrations. In the weaker dilutions, the rate was only slightly increased, followed by depression, whereas the stronger solutions all caused a depression of rate. Further observations were made with stronger solutions of sulfanilamide, up to 0.8%, essentially with the same results. The heart always stopped in diastole. This was not due to the weight of the lever, since it also stopped in diastole when wholly disconnected from the lever.

In order to determine if any part of the action of sulfanilamide was caused by a change in pH, both the Ringer's solution and the 0.5% solution of sulfanilamide in Ringer's were tested. The sulfanilamide raised the pH very little so that the solution was still within the pH range permissible for frog heart Ringer's. Therefore, it was not a change in pH which affected the heart.

The effect of sulfanilamide on the isolated frog heart is stimulation followed by depression. The stimulation increases the amplitude. The effect is transient, however, and often lasts only 10 minutes although, in some cases, it persists as long as 20 or 30 minutes. The maximum increase occurs with a 0.25% solution. The rate may also be slightly increased but it is the amplitude that shows the greatest effect of stimulation. On the other hand, the secondary depression appears as a decreased rate. In the lower concentrations (up to 0.2%) this is not evident in the first 10 minutes but in higher concentrations, may set in sooner. The depressive effect increases with the concentration of sulfanilamide. A 0.5% solution of sulfanilamide causes a 50% to 100% decrease in rate within 10 minutes after it is introduced into the heart. While some hearts stopped within the first 10 minutes, others maintained a very slow rate with some irregularity for 40-60 minutes.

10892 P

Reduction in Experimental Rat Caries by Fluorine.

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Dental research in the field of caries prevention received new impetus when Hoppert, Webber and Canniff[1] experimentally produced dental caries in 90 to 100% of their rats by feeding them a diet, which although adequate nutritionally, consisted mainly of coarse corn particles. Their hypothesis was that the corn particles became impacted in the teeth and produced decay. Lilly[2] obtained a 90% reduction in the incidence of dental decay by substituting commercial casein for the powdered whole milk (20% of the Hoppert, Webber and Canniff diet). The same year, Hodge, Luce-Clausen and Brown[3] found that the commercial casein used by them was contaminated with fluorine (0.2%). It occurred to us that fluorine might be the factor producing the caries reduction in Lilly's experiments. Two major findings in the literature support this contention: (1) clinical observations by Dean and others[4] give evidence of a reduction of caries in areas of endemic fluorisis (mottled enamel); (2) analyses made by Armstrong and Brekhus[5] have shown that sound enamel contains a higher percentage (.0111%) of fluorine than carious enamel (.0069%). Our experiment was, therefore, undertaken to compare the incidence of caries on the Hoppert, Webber and Canniff diet with (a) that on a diet in which "fluorine-free" casein is substituted for the powdered whole milk, and (b) that on the latter diet to which fluorine is added. While this experiment was in progress, Miller[6] reported that adding fluorine or iodoacetic acid to a modified Hoppert, Webber and Canniff diet markedly reduced the incidence of caries.

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[1] Hoppert, C. A., Webber, P. A., and Canniff, T. L., *Science*, 1931, **74**, 77.

[2] Lilly, C. A., PROC. SOC. EXP. BIOL. AND MED., 1938, **38**, 398.

[3] Hodge, H. C., Luce-Clausen, E. M., and Brown, E. F., *J. Nutrition*, 1939, **17**, 35.

[4] Dean, H. T., *Public Health Rep.*, 1938, **53**, 1443.

One hundred and thirty-five stock rats at weaning age were divided into 3 groups, separating littermates and segregating the sexes. The first group of 45 rats received the Hoppert, Webber and Canniff diet consisting of 60% coarse corn meal, 30% powdered whole milk, 6% linseed meal, 3% alfalfa meal and 1% NaCl (with added vitamins). The second group received essentially the same diet with casein substituted for the powdered whole milk. The third group received the latter diet plus the daily oral administration of a drop of an aqueous solution of KF containing 3 mg of fluorine. Each of the 3 groups of rats was divided into 3 sub-groups. The first received corn particles considerably larger than 20 mesh, the second was given particles between 10 and 20 mesh, and the third unsifted or run of the mill cracked corn. The different corn sizes were employed to establish whether caries incidence increased with particle size or whether approximately 20 mesh corn size was optimum. The food and distilled water were given *ad lib.* The rats were weighed every 4 days and the teeth observed periodically, using Hunt's technic (personal communication). After 200 days on the diets, the animals were sacrificed and the separated jaws examined under binoculars (15 ×). Missing cusps and macroscopic caries were recorded. In several cases it was difficult to distinguish between true fractures and severe attrition.

As may be seen from Table I, the rats receiving powdered milk or casein had about equal incidence of caries and an equal number of cusps involved. The rats receiving fluorine showed a 60 to 70% decrease in both cavities and cusps involved, as compared to the other 2 groups. Thirteen of the 42 rats receiving fluorine had perfect teeth; in contrast, all of the rats in the other 2 groups had caries. In all the rats, upper teeth were more involved than lower, thus, the upper teeth had 171 cavities with 620 cusps involved, while the

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[5] Armstrong, W. D., and Brekhus, P. J., *J. Dent. Res.*, 1938, **17**, 393.

[6] Miller, B. F., PROC. SOC. EXP. BIOL. AND MED., 1938, **39**, 389.

TABLE I.
Effect of Fluorine on Caries Incidence.

Diet constituent varied	No. rats	No. cavities per rat, av. ± s.d.	No. cusps involved per rat, av. ± s.d.	No. caries-free rats
Powdered milk	40	3.5 ± 1.2	10.6 ± 4.5	0
Casein	42	3.0 ± 1.1	9.0 ± 3.3	0
Casein and fluorine	42	1.1 ± 1.0	3.1 ± 3.6	13

lower teeth had 145 cavities with 360 cusps involved. The variation of corn particle size was found to be unimportant; this was to be expected since all the diets contained particles larger than 20 mesh.

There is little doubt that fluorine not casein is responsible for the diminished incidence of fractured and carious teeth. Therefore, the caries reduction reported by Lilly can probably be attributed to fluorine contamination of the casein which he fed.

10893

Acceleration of Hemolysis in Relation to Chemical Structure. II. The Straight-Chain Alcohols.

ERIC PONDER AND CHESTER HYMAN.

From the Biological Laboratory, Cold Spring Harbor, L. I.

One of us (Ponder[1]) has already discussed the relation between the chemical structure of the benzene derivatives and the extent to which they accelerate hemolysis by the simple lysins, and has formulated a single expression to indicate the accelerating power of any substance in any concentration.* This note considers acceleration by the straight chain alcohols, from propyl to nonyl.

Method. The method used was that described by Ponder.[1] By a comparison of 2 time-dilution curves the acceleration for a given concentration of alcohol was determined. From this R value and the concentration of alcohol in mM/1, a value for (1-R)/c was obtained.†

As with the benzene derivatives, the solubility of the higher alcohols in water is very small and great care has to be taken to obtain known amounts in solution. In all cases below amyl alcohol the concentration employed was as close to saturation as possible. Propyl alcohol showed no acceleration in concentrations as great as 1.5 M/1. In higher concentrations, about 5 M/1, this alcohol is lytic *per se* (Gordon[2]). Butyl and amyl alcohols are relatively soluble in water and it was unnecessary to approach saturation to get sufficiently concentrated solutions for acceleration. The concentrations used are given in Column 2 of Table I.

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[1] Ponder, E., *J. Exp. Biol.*, 1939, **16**, 38.

* Published in *J. Exp. Biol.*, 1939, **16**, 38.

† Previously we have used (R-1)/c as a measure of acceleration or inhibition. Here we use (1-R)/c as a measure of acceleration in order to avoid obtaining logarithms of negative values. This is merely a mathematical transformation.

TABLE I.

Alcohol	Concentration mM/1	R	(1-R)/c
Propyl	1,500	.99	—
Butyl	250	.67	.0014
Amyl	100	.38	.0062
Hexyl	5	.875	.025
Heptyl	2	.85	.075
Caprylic	2	.774	.116
Nonyl	0.5	.506	.988

The alcohols used were obtained from Eastman Kodak Company, and had boiling-point ranges of not more than 5°C. Saponin in dilutions of 1:10,000 to 1:60,000 was used as the lysin throughout. Time-dilution curves were plotted at 25°C in isotonic NaCl.

Results. In Table I the results with the various alcohols are summarized.

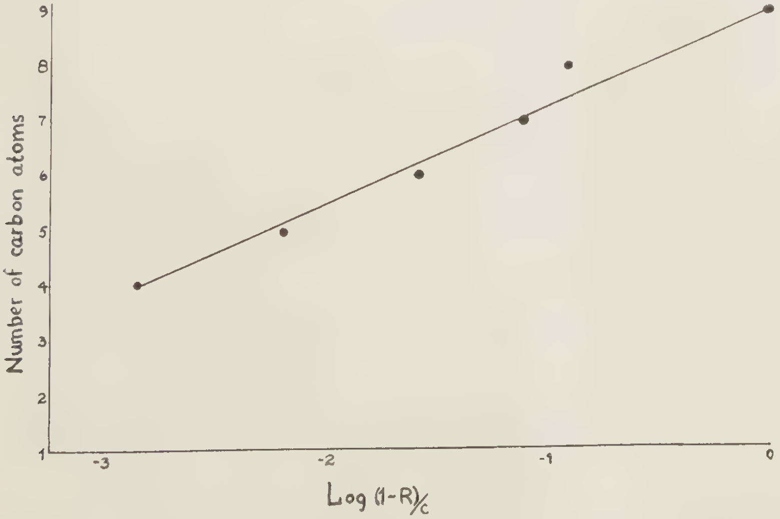

FIG. 1.

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[2] Gordon, A. S., *J. Exp. Biol.*, 1932, **9**, 336.

In Fig. 1 the logarithm of (1-R)/c is plotted against the number of carbon atoms in the alcohol. The resulting curve approximates to a straight line. The slope of this line is approximately 1.75; it intersects the zero of the logarithmic scale at approximately 9 carbons. Using N for the number of carbon atoms, and A to replace (1-R)/c the equation of the line becomes:

$$N = 1.75 \log A + 9,$$

so that there is a logarithmic relation between the number of carbon atoms in any straight chain alcohol and its power as an accelerator of hemolysis. There is some suggestion here of a relation to Traube's Rule, the acceleration being connected with an accumulation of the alcohol in the surface. Such an accumulation occurs in the case of acceleration of the benzene derivatives, but the methods at our disposal, when applied to such dilute solutions, make it impossible to decide the matter one way or the other.

10894

Uterine Distention and Maintenance of Pregnancy Following Oöphorectomy in the Rat.*

Hans O. Haterius and M. J. Kempner.

From the Departments of Physiology, Wayne University College of Medicine, Detroit, and Long Island College of Medicine, Brooklyn, N. Y.

One of the writers has reported[1] that removal of ovaries in the rat need not result in interruption of pregnancy, provided but one fetus be retained and that the placentae of removed fetuses be left *in situ*. On the basis of this finding, it was suggested that the placentae exercise some degree of endocrine function in the maintenance of gestation—not so well developed, perhaps, as in certain monotocous forms in which the ovaries may be dispensed with during an appreciable part of pregnancy, but sufficiently so that a number of placentae can maintain the gestation of a single surviving fetus; for further discussion reference may be made to the original paper.

Now, in view of the striking effects on uterine growth and development that have been reported to follow experimental distention[2, 3, 4] the suggestion was advanced[4] that distention might prove a factor in maintenance of pregnancy under such experimental conditions; the investigation of this possibility has led to the present report.

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* The experimental work was carried out at the Biological Laboratory, Cold Spring Harbor, Long Island, N. Y.

[1] Haterius, H. O., *Am. J. Physiol.*, 1936, **114**, 399.

The procedure followed was similar to that employed in the earlier work. That is, rats were mated and at a definite time in pregnancy (13th-18th day) were subjected, under ether anesthesia, first to unilateral oöphorectomy and removal of fetuses and placentae in excess of one, followed in 2 days by extirpation of the second ovary; in our experience one-stage operations almost invariably result in abortion, as exemplified by the last 4 cases in Table I. In fetal removal small incisions at the implantation sites allowed for "shelling out" of uterine contents, which then were replaced by equivalent masses either of melted paraffin (m.p. 40° C) or of malleable wax. The incisions were closed, and observations were made at suitable times.

TABLE I.
Summary of Cases of Uterine Distention and Fetal Retention.

Animal	Laparotomy	Oöphorectomy	Results
1	14[*]	16	22: fetus alive, 3.6 g
2	14	16	22: ” ” 5.36 ”
3	14	16	22: ” ” 4.98 ”
4	15	17	23: ” dead, 5.35 ”
5	15	17	22: ” alive, 3.48 ”
6	13	15	22: ” ” 3.43 ”
7	15	17	22: ” dead, 4.1 ”
8	16	18	23: ” ” 4.8 ”
9	15	17	22: ” ” badly crushed
10	14	16	22: ” alive, 3.3 g
11	11-13[†]	One stage operation	23: fetal mass in uterus
12	11-13[†]	” ” ”	22: uterus empty
13	17-18[†]	” ” ”	22: fetal mass in advanced dissolution
14	17-18[†]	” ” ”	22: uterus empty

[*] Figures in this and succeeding columns refer to day of operation or autopsy.
[†] Stage of pregnancy estimated at time of laparotomy.

In no case did delivery occur, either at normal term or later, and autopsies were performed on the 22nd or 23rd days, as indicated in Table I. In a series of 10 animals, fully developed fetuses were recovered, 6 alive and apparently normal. Careful dissection failed to reveal accessory or residual ovarian tissue. In 4 instances (Cases 11-14), in which both ovaries were removed at the time of fetal

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[2] Van Dyke, H. B., and Gustavson, R. G., *J. Pharmacol. and Exp. Therap.*, 1929, **37**, 379.

[3] Markee, J. E., Wells, W. M., and Hinsey, J. C., *Anat. Rec.*, 1935, **64**, 221.

[4] Reynolds, S. R. M., *Cold Spring Harbor Symposium on Quant. Biol.*, 1937, **5**, 84.

expulsion, pregnancies were interrupted, despite a maintained uterine distention.

It would appear, therefore, that maintenance of adequate uterine distention may contribute to the preservation of pregnancy in the experimentally monotocous and oöphorectomized rat. While this does not invalidate the possibility of an endocrine activity of retained placentae, especially in view of the results reported by Kirsch,[5] it demonstrates that a mechanical factor may be of importance, although the physiological mechanism is as yet speculative. It seems probable that mechanical and hormonal factors work together; Kirsch, for example, has shown that pellets inserted in the completely evacuated uterus of the oöphorectomized rat are expelled within 40-48 hours, whereas if some placentae are allowed to remain, either in the same or in the contralateral horn, both placentae and pellets are retained until the time of normal term—indicative of a contribution by the placentae to progestational factors.

Growth influences exerted by distention are well known. For example, (a) the occupied horn in a unilateral pregnancy is reported to be larger than the empty horn,[6] although both are presumably under identical hormonal influences; (b) local uterine growth has been obtained by insertion of rolled rubber dams during pseudopregnancy;[2] (c) both fetal number and fetal weights are important factors;[7] (d) marked uterine growth occurs as a result of fluid distention,[3] and (e) growth, moreover, still occurs in a uterine horn after interruption of its thoraco-lumbar visceral innervation.[8] Finally, Reynolds, in a series of papers (summarized in [4]) has reported the effects of uterine distention by means of pellets in the oöphorectomized rabbit, both untreated and subjected to hormone administration. In the course of this work it was demonstrated that the distention factor will operate in the absence of ovarian hormones and that both hypertrophy and hyperplasia will occur under this circumstance.

An explanation of the results reported here is by no means clear, although it is tempting to suggest that fetal integrity was preserved by the combined influences of the retained placenta and the mechanical effects of the distending pellets serving to prevent retrogression of the progestational changes incident to pregnancy or, perhaps, to prevent an increase in uterine motility.[4] Maintenance of more adequate vascular relations may also have been involved. Obviously, these possibilities are as yet conjectural.

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[5] Kirsch, R. E., *Am. J. Physiol.*, 1938, **122**, 86.

[6] Knaus, H. H., *Münch. med. Wochenschr.*, 1929, **10**, 404.

[7] Markee, J. E., and Hinsey, J. C., *Anat. Rec.*, 1935, **61**, 311.

[8] Hinsey, J. C., and Markee, J. E., *Anat. Rec.*, 1935, **61**, 253.

10895

Sulfapyridine Potentiation of Narcotic and Toxic Effects of Papaverine* in Rats and Rabbits.

SUSI GLAUBACH. (Introduced by W. Antopol.)

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In the course of investigating the effect of papaverine on the blood pressure of rabbits treated with sulfapyridine,‡ [1] it was observed that, in these animals, papaverine had a profound narcotic effect. This finding is significant since the literature refers only to the sedative action of papaverine, Schroeder[2] reported that the oral administration of 0.5 g of papaverine produced drowsiness only, and that 1.0 g produced sleep and catatonia in ¼ to ½ hour. Deep narcosis was not observed even with 2.0 g, and increased excitability, rather than sedation, ensued. This was evidenced by shivering, convulsions, dyspnoea, dilation of the pupils and finally arrest of respiration and death. Similar findings were obtained by Zeelen[3] after the subcutaneous injections of papaverine in normal rabbits. He also was unable to produce deep narcosis.

The present investigation deals with the effect of papaverine on rabbits and rats to which sulfapyridine had been previously administered. The animals were first tested for their reaction to papaverine alone. After a rest period of 6-8 days the papaverine was injected subcutaneously 3 hours after the oral administration of sulfapyridine. Eight to 10 days later the animals which survived the second set of experiments were again retested for the papaverine response alone.

Rat Experiments. In this series 10 male rats weighing between 120-200 g were used. All of the animals received a subcutaneous injection of 0.3 mg papaverine per gram of rat. Sedation, diminished tonus of the muscles of the hind limbs, and catatonia were observed in 15-20 minutes in 7 of the animals. These effects lasted for about 1 hour and then rapidly disappeared. The remaining 3 rats showed no detectable nervous effect. Six days later the animals were fed 4 mg sulfapyridine per gram body weight, followed in 3 hours by 0.3 mg papaverine per gram body weight subcutaneously. The exaggeration of the papaverine effect was striking. In 5-15 minutes all the rats were in deep anesthesia. In 3 rats, the narcotic effect was interrupted by brief convulsions. Six of the animals died in 15 to 36 minutes. In these, breathing was first accelerated, then shallow, later irregular, and finally ceased while the heart continued to beat. If artificial respiration was administered, the heart beat could be maintained for 10-15 minutes, after which it became weak and slow, and finally ceased. At necropsy, the heart was found to have stopped in diastole.

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* The term papaverine is used to designate Papaverine Hydrochloride.

‡ We wish to thank Dr. J. J. Carlisle of Merck & Co. for supplying the sulfapyridine used in this investigation and to express our appreciation to Miss Bessie Zirin for her technical assistance.

[1] Work in progress in collaboration with W. Antopol.

[2] v. Schroeder, A., *Arch. f. exp. Path.*, 1883, **30**, 125.

[3] Zeelen, B., *Z. f. exp. Path. u. Ther.*, 1911, **8**, 590.

The 4 surviving rats recovered from the deep narcosis in 3-4 hours. Ten days later these animals were again tested for papaverine effect alone, at which time they showed no observable nervous manifestations whatsoever.

Rabbit Experiments. These experiments were similar to those performed on the rats. Nine rabbits weighing 1600-2000 g were used. Seven of these received a subcutaneous injection of 0.15 g, one 0.2 g, and one 0.04 g papaverine per kilo body weight. The representative effects are exemplified in rabbit No. 1603. After sulfapyridine, the papaverine acted not only more profoundly, but also started earlier and lasted longer than with papaverine alone in the initial experiment. Eight days later the experiment was again repeated with the sulfapyridine and papaverine, with identical results. After a rest of 8 more days, papaverine alone again was employed, without any recognizable nervous manifestations. Since it is reported that the effect of papaverine is diminished after repeated administration, it is likely that here also the absence of papaverine effect when administered alone in the final phase of the experiment was also dependent upon an increased tolerance to it. To substantiate this, after another rest period papaverine was again administered with sulfapyridine and a repetition of its profound toxic effect and death of the animal resulted.

In the other 8 rabbits, there was also a pronounced potentiation of the papaverine effect when it was administered after the sulfapyridine. Three rabbits died when the papaverine was given after the sulfapyridine. After 8 days the surviving 5 rabbits were tested with the papaverine alone and showed slight or no effect.

The animal which received 0.04 mg papaverine per kilo body weight, a dose which produces no demonstrable nervous effect, also showed a definite papaverine effect when administered after sulfapyridine.

It remained to be determined whether papaverine, due to its dilating effect on the splanchnic vessels, caused concomitant increase in sulfapyridine absorption, thus giving the added effect, or whether the central nervous system was sensitized for papaverine by the sulfapyridine. With sulfapyridine in non-fatal doses no nervous symptoms have been observed,[4] in contrast to sulfanilamide which produces ataxia and intoxication.[5-8] Sulfapyridine determinations were made in the blood after administration but before papaverine injection, at the height of the narcosis after papaverine, during recovery, and when the animal appeared normal. The total sulfapyridine was found to be higher during the phase of recovery than during the narcosis, and even when the animal appeared to have recovered completely, the total sulfapyridine in the blood was 75% higher than at the height of the narcosis. It may be concluded, therefore, that the sulfapyridine appeared to have a latent effect which was only detectable after papaverine was administered and that the deep anesthetic effects were not dependent upon increased blood sulfapyridine.

This finding may be of importance in cases in which patients receive alkaloids which affect the nervous system. Investigations with codeine, morphine, soporifics and analgesics as well as similar studies with sulfanilamide are now under investigation.

It is interesting to note that apparently more sulfapyridine becomes esterified under the effect of papaverine. This may be of interest in view of the finding of acetylated sulfapyridine urolithiasis in animals[9] The influence of various medications on the esterification of sulfapyridine also is being investigated.

Summary. 1. Sulfapyridine potentiates the anesthetic action of papaverine. 2. This is not dependent upon an increased concentration of sulfapyridine in the blood.

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[4] Molitor, H., and Robinson, H., *J. Pharm. and Exp. Therap.*, in press.

[5] Halpern, B. N., and Meyer, R. L., *Presse Med.*, 1937, **45**, 747.

[6] Marshall, E. K., Jr., Cutting, W. C., and Emerson, K., Jr., *J. A. M. A.*, 1938, **110**, 252.

[7] Wien, R., *Quart. J. Pharm.*, 1938, **11**, 217.

[8] Molitor, H., and Robinson, H., *J. Pharm. and Exp. Therap.*, 1939, **65**, 405.

[9] Antopol, W., and Robinson, H., PROC. SOC. EXP. BIOL. AND MED., 1939, **40**, 428.

10896

Phenanthrene in Relation to Growth of Rats Maintained on a Low-Casein Diet.

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It was shown that halogenated benzenes and naphthalene, when fed to growing rats which were maintained on a diet low in casein but sufficient for moderate growth, check the growth. Upon incorporation of cystine, methionine or glutathione into the diet which contained either halogenated benzenes or naphthalene, the growth was resumed. These results were interpreted as indicating that the halogenated benzenes and naphthalene induced cystine deficiency in the rat which was alleviated by the administration of the sulfur-containing amino acids. This interpretation was supported by the fact that the halogenated benzenes and naphthalene are known to yield in the rat the corresponding mercapturic acids.[1, 2]

Some time ago evidence was presented to the effect that phenanthrene, when fed to dogs, might be excreted in the urine as a mercapturic acid.[3] It appeared of interest to investigate the effect of phenanthrene on growth of rats which were kept under the experimental conditions referred to above.[1, 2] Should the effect of phenanthrene on growth of rats be similar to that produced by halogenated benzenes and naphthalene it would, perhaps, be justifiable to assume that phenanthrene, like anthracene,[4] is detoxicated in the rat to yield, in part, a mercapturic acid. The present study was therefore undertaken.

The general experimental procedure used in the present study was similar to that employed previously.[1, 2] Two litters of albino rats, 30 days old, were used. The composition of the diet (referred to in the table as Diet C-6) was as follows: Casein, 6 parts; corn starch, 50; sucrose, 15; Osborne and Mendel[5] salt mixture, 4; and Crisco, 25. In addition to the diet each rat received daily 400 mg of dry yeast and 100 mg of cod liver oil. Phenanthrene was fed in doses of 0.500 g per 100 g of the diet. 0.500 g of l-cystine, 0.640 g of dl-methionine, or 0.500 g of taurine per 100 g of the diet was fed as

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[1] White, A., and Jackson, R. W., *J. Biol. Chem.*, 1935, **111**, 507.

[2] Stekol, J. A., *J. Biol. Chem.*, 1937, **121**, 87; 1937-38, **122**, 55.

[3] Stekol, J. A., PROC. SOC. EXP. BIOL. AND MED., 1935, **33**, 170.

[4] Boyland, E., and Levi, A. A., *Biochem. J.*, 1936, **30**, 1225.

[5] Osborne, T. B., and Mendel, L. B., *J. Biol. Chem.*, 1919, **37**, 572.

supplements to the diet which contained phenanthrene. For the sake of economy of space the data are presented in summarized form in Table I.

TABLE I.
Effect of Phenanthrene on Growth of Rats Fed Diet C-6 With or Without l-cystine, dl-methionine, or taurine.*

Rat	Food intake per day g	Supplement per 100 g diet g	Phenanthrene per 100 g diet g	Days on diet	Initial wt, g	Total gain, g
1 ♂	6.6	0	0	40	48	+33
	5.1	0	0.5	14	81	— 5
	7.4	0.5 cystine	0.5	14	76	+16
	6.0	0	0.5	14	92	— 2
	7.7	0.64 methionine	0.5	14	90	+12
2 ♀	6.2	0	0	40	44	+39
	4.8	0	0.5	14	83	— 9
	6.8	0.5 cystine	0.5	14	74	+15
	5.5	0	0.5	14	89	— 5
	7.4	0.64 methionine	0.5	14	84	+16
	7.7	0	0	14	100	+12
3 ♂	5.8	0	0	40	49	+32
	5.0	0	0.5	14	81	— 5
	4.8	0.5 taurine	0.5	14	76	— 7
	7.8	0.64 methionine	0.5	14	69	+16
	6.6	0	0.5	14	85	— 2
	7.2	0.5 cystine	0.5	14	83	+14
	7.5	0	0	14	97	+10
4 ♀	5.7	0	0	40	46	+40
	5.5	0	0.5	14	86	— 6
	6.6	0.5 cystine	0.5	14	80	+10
	6.0	0.5 taurine	0.5	14	90	— 2
	7.0	0	0	14	88	+14
	6.3	0	0.5	14	102	— 5
	7.9	0.64 methionine	0.5	14	97	+12

* This data for each rat presented in this table are representative of experiments obtained on 4 animals.

The results presented indicate that phenanthrene, when fed to growing rats, checks the growth. The growth is resumed upon the administration of l-cystine, dl-methionine but not taurine. In this respect phenanthrene is similar to halogenated benzenes or naphthalene. Pending the isolation of detoxication products of phenanthrene from the urine of rats which were fed the hydrocarbon, it is assumed that the cessation of growth of rats on phenanthrene diet is induced by the depletion of cystine reserves by phenanthrene for detoxication purposes. Apparently the detoxication of phenanthrene in the growing rat takes precedence over the needs of the animal for growth purposes.

Summary. 1. Phenanthrene was fed to growing rats which were maintained on a low-casein diet. The growth ceased. It was resumed upon incorporation, into the phenanthrene diet, of l-cystine, dl-methionine but not taurine. 2. Assumption is made that phenanthrene is detoxicated in the rat to yield a mercapturic acid, the formation of which is responsible, at least in part, for the cessation of growth of rats ingesting phenanthrene.

10897 P

Identity of "Inhibitor" and Antibody in Extracts of Virus-Induced Rabbit Papillomas.

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Saline extracts of the virus-induced papillomas of domestic rabbits often contain something that inhibits or neutralizes the virus *in vitro*, as Shope first noted.[1] Serological studies have shown that when mixed with it the blood of rabbits carrying these papillomas also has in most instances the power to neutralize the virus *in vitro*.[2] Experiments were undertaken to learn whether the "inhibitor" procured from the papillomas may not be specific antiviral antibody of the sort present in the blood.

To test for "inhibitor", 10% extracts of freshly procured papillomas were prepared by grinding in sand, suspending in saline, and centrifuging at about 4400 rpm for 20 minutes in an angle-head centrifuge. The clear supernatant fluids were then mixed in equal parts with a Berkefeld filtrate of the virus-induced growths of cottontail rabbits, containing virus of known titer, incubated 2 hours at 37°C, and rubbed into scarified areas on the skin of normal domestic rabbits according to a titration technic already described.[2]

The papillomas of 13 domestic rabbits with high serum-antibody titers, as determined by virus-neutralization and complement-fixation tests,[2, 3] all yielded large amounts of the "inhibitor", extracts of the growths neutralizing completely or almost completely an amount of virus equal to 500 minimal infective doses. Similar growths of 6 domestic rabbits which had but little circulating antibody yielded little or none of the "inhibitor", the extracts of these, prepared in the same way, having practically no capacity to neutralize virus.

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[1] Shope, R. E., *J. Exp. Med.*, 1933, **58**, 607.

[2] Kidd, J. G., Beard, J. W., and Rous, P., *J. Exp. Med.*, 1936, **64**, 63, 79.

[3] Kidd, J. G., *J. Exp. Med.*, 1938, **68**, 703, 725, 737.

To study further the relation between the "inhibitor" and antibody, pieces of the papillomas of 4 domestic rabbits, all produced by the same inoculum, were repeatedly removed and tested for yield of "inhibitor", the amount of antibody in the serum of the rabbits being determined concurrently. On the 21st day after inoculation with the virus, that is to say, only 7 to 10 days after the growths had appeared and before antibody could be detected in the blood in noteworthy amount, the papillomas yielded little or no "inhibitor". Later tests (37th and 111th days) showed that, as the serum-antibody titer of 3 of the rabbits rose, the amount of inhibitory substance that could be procured from the growths increased in direct proportion to the amount of antibody present in the serum. The fourth animal failed to develop any noteworthy amount of circulating antibody and the papillomas yielded none of the "inhibitor" in any test. Further experiments showed that when the production of humoral antibody was stimulated by intraperitoneal injections of a papilloma-virus suspension the amount of inhibitory substance procurable from a given papilloma underwent a corresponding increase.

None of the inhibitory substance could be detected in the sera or in saline extracts of the skin, muscle, and liver of 4 normal rabbits; nor did extracts of the Brown-Pearce tumor, which had grown in 2 rabbits uninfected with the papilloma-virus, exert any effect upon the latter. In contrast to these findings the "inhibitor" was present in extracts of the skin, muscle, and liver of 6 rabbits carrying papillomas and having considerable amount of antibody in their serum. The neutralizing effect of extracts of papillomas was regularly greater than that of extracts of other tissues from the same rabbit, but much less than that of a 10% dilution of its serum.

The "inhibitor", like the antiviral antibody,[2] exerts no discernible influence on the course of the papillomas, as repeated observations have demonstrated, these enlarging progressively or dwindling and vanishing, irrespective of their content of "inhibitor". Nor is antibody or "inhibitor" primarily involved in the "masking" of the virus in the papillomas of domestic rabbits, for these have failed to yield active virus on extraction even when little or no antibody was present in the blood of the hosts bearing them[4] and when no "inhibitor" could be detected in them.

All of the findings indicate that the "inhibitor" present in extracts of the virus-induced rabbit papillomas is actually specific antiviral antibody. They raise the question whether the "inhibitor" found in extracts of chicken sarcomas[5] may not be of similar nature.

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[4] Kidd, J. G., *J. Exp. Med.*, 1939, **70**, in press.

10898

Decreased Tolerance of Mice to Intraperitoneal Glucose Injections in Certain Neurotropic Virus Infections.*

ANSON HOYT, MARGARET HOLDEN AND RUTH A. RAWSON.

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Certain neurotropic virus infections of mice cause a definite decrease in the tolerance of these animals to intraperitoneally injected glucose.

Glucose was dissolved in distilled water and injected intra-abdominally into white Swiss mice in constant volumes of 0.05 cc per gram of body weight. The concentrations of glucose employed varied from 45 to 10%. Viruses were inoculated intracerebrally and test injections of glucose were generally given during the early stage of disease symptoms, normal controls being included whenever virus-infected mice received dextrose. The viruses utilized were rabies fixed virus, a neurotropic strain of herpes, St. Louis encephalitis and equine encephalomyelitis (Western strain). The work included in Table I covers many small experiments, all of which pointed in the same direction.

Table I indicates that 25 and 20% glucose killed almost all virus-infected mice, whereas most corresponding controls survived. The injection of glucose, in the case of those mice that survived, had no effect on the subsequent course of the above virus diseases; the surviving virus-infected mice developed symptoms of increasing severity and eventually died.

Intraperitoneal injections of glucose often cause immediate symptoms of peritoneal irritation which quickly subside, after which the mice appear normal for 15 minutes or more. They then act irritable,

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[5] Sittenfeld, M. J., Johnson, B. A., and Jobling, J. W., PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 517; Murphy, J. B., and Sturm, E., *Science*, 1931, **73**, 266; Claude, A., *Am. J. Cancer*, 1939, **37**, 59.

* This research was supported by a grant from the W. J. Matheson Fund for the study of encephalitis.

TABLE I.

Fate of Mice Receiving Intraperitoneal Glucose.

% of glucose	Normal control mice		Mice Showing Symptoms of							
			Rabies		Herpes		St. Louis encephalitis		Equine encephalomyelitis	
	Died	Survived	Died	Survived	Died	Survived	Died	Survived	Died	Survived
45	6	0	—	—	—	—	—	—	—	—
40	17	1	1	0	—	—	2	0	—	—
35	14	4	1	0	1	0	—	—	—	—
30	9	11	4	0	2	0	6	0	2	0
25	2	19	8	1	2	0	6	1	2	1
20	1	21	19	0	2	2	7	1	2	1
15	0	7	6	3	0	2	4	1	0	2
10	0	5	3	4	—	—	—	—	—	—

— = No mice injected.

All mice received intraperitoneal glucose in volumes of 0.05 cc per gram of body weight.

their fur is ruffled, breathing becomes irregular, they show increasing cyanosis, tremors and convulsions develop and death ensues, usually within an hour. Sublethal doses of glucose may cause various symptoms of distress followed by recovery; still smaller doses produce no symptoms. Autopsy of both normal and virus-infected mice, that have died, shows a very constant picture. There is a considerable amount of free peritoneal fluid, sometimes blood-tinged. The abdominal viscera are hyperemic. Most extra-abdominal areas appear dehydrated and section through the chest causes little bleeding. The brain, however, exhibits marked gross hyperemia, a fact that was verified by several microscopic sections.

An important factor in explaining the decreased tolerance to intraperitoneal dextrose, that is shown by virus-infected mice, would appear to be the dehydration and presumably also demineralization that is produced by the osmotic effect of glucose in the peritoneal cavity. The following experiments involve only rabid and normal animals.

Mice that have developed symptoms of rabies stop drinking and rapidly become dehydrated. Intraperitoneal glucose produces considerable dehydration in normal mice and accentuates the dehydration that is already present in rabid animals. Red cell counts were performed on each of a number of mice to ascertain the amount of dehydration that had occurred. Blood was obtained by cutting the throats of the mice and collecting a few drops in a Petri dish; the animals were then immediately killed. The mean red cell count of 12 normal mice was 8,900,000 (limits 8,000,000 and 11,100,000), whereas the mean count of 12 rabid mice was 11,800,000 (limits 9,900,000 and 13,700,000). Six rabid mice, injected intraäbdominally with 20% glucose and bled during terminal convulsions, showed a mean count of 13,400,000 (limits 11,900,000 and 15,300,000). Six normal mice were injected with 20% glucose; they showed no symptoms but were bled one hour later, an interval roughly corresponding to the time required by rabid mice to die following intraperitoneal administration of 20% dextrose. Their mean count was 11,200,000 (limits 9,800,000 and 12,500,000). Six normal mice that received 40% glucose were bled during terminal convulsions. They showed a mean count of 11,400,000 (limits 10,500,000 and 12,000,000).

An attempt was made to alter the susceptibilities of normal and rabid mice to 20% intraperitoneal glucose. Fifteen normal mice were dehydrated by withholding water for 42 hours and were then injected with 20% glucose. Three died after typical convulsions. The remainder survived but all showed definite symptoms of distress which often progressed as far as tremors and mild convulsions. Fifteen mice in early stages of rabies were given 4 cc of Ringer's solution (without glucose) subcutaneously, one evening, and received an additional 5 cc the next morning. This was followed in 2 hours by 20% intraperitoneal glucose. Five of these mice developed severe convulsions and died but the remainder, after showing symptoms of varying severity, survived. In this manner, by altering the factors of dehydration, it was possible to approximate the susceptibilities of rabid and normal mice to intraperitoneal injections of dextrose.

Many diseases may alter the host's ability to metabolize glucose, a fact that has been demonstrated in experimental poliomyelitis.[1] Such alterations in dextrose metabolism do not appear, however, to play a major rôle in accounting for the different susceptibilities of rabid and normal mice to intraperitoneal injections of glucose. Both groups have essentially the same tolerance for dextrose when it is given intravenously. Glucose was injected into the tail veins of both rabid and normal mice in volumes of 0.02 cc per gram body weight. Five normal mice received 45% glucose, a concentration which proved too high, all rapidly had severe convulsions and died. Forty percent glucose killed 3 out of 6 normal mice and 1 out of 4 rabid mice, while 35% glucose killed 1 out of 6 normal mice and 1 out of 5 rabid mice.

Determinations were made of the actual blood glucose levels obtained by intraperitoneal dextrose injections. The mice were anesthetized with nembutal, their throats were cut and blood was collected in a watch glass and mixed with crystals of potassium oxalate. The blood volumes employed were generally 0.1 cc and determinations were run by the micro Schaeffer-Hartmann method. Preliminary experiments showed that nembutal anesthesia had little effect on the blood glucose level. Six starved normal mice showed blood glucose levels ranging between 124 and 150 while 4 rabid mice varied from 90 to 134 mg per 100 cc. Five rabid mice received 20% glucose and blood was taken about 1 hour later during terminal convulsions; their mean blood glucose level was 766 mg per 100 cc (limits 594 and 864). Five normal mice, which also received 20% glucose and remained symptom-free up to one hour, were killed; their mean blood glucose was 722 (limits 668 and 800). It thus appears that 20% intraperitoneal glucose, although it killed rabid mice while producing no symptoms in normal animals, built up essentially the same blood glucose levels, in a constant time, in both groups.

Five normal mice received 40% glucose and were bled during terminal convulsions. These animals showed a mean blood glucose level of over 1420 mg per 100 cc (limits 1334 and 1500+), a level far exceeding that of the rabid mice which died following administration of 20% glucose. This level, although high, is presumably only one factor in causing the death of these normal animals, dehydration being another such factor. Wierzuchowski[2] has reported that the blood glucose level of dogs may be brought up to over 3500 mg per 100 cc before death occurs.

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[1] Jungeblut, C. W., and Resnick, R., *Am. J. Dis. Child.*, 1936, **51**, 91.

Summary. Mice in the symptomatic stages of various neurotropic virus infections are more susceptible than normal mice to the lethal effect of intraperitoneally injected glucose. The dehydration produced by intraperitoneal glucose appears to be a major factor in this differential susceptibility.

10899

Effect of Adrenalectomy on Fat Absorption Measured by Fat Excretion in the Stool.

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Contrary to the view of the Verzar group,[1, 2, 3] Barnes, *et al.*,[4] concluded that adrenalectomy has no significant effect on the absorption of fat in the form of corn oil or the methyl esters of the fatty acids of corn oil. The experiments comprising this report represent a corollary of the earlier work. In the experiments of Barnes and coworkers, fat absorption was measured in the usual way and in what might be termed an acute manner. A single large dose of fat was administered 4 days after adrenalectomy. In the present experiments, fat absorption was studied over a longer time, as a matter of fact, over a period of days, after removal of the glands and under ordinary conditions of nutrition through the simple expedient of following the excretion of fat in the stools in relation to the amount of

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[2] Wierzuchowsky, M., *J. Physiol.*, 1936, **87**, 311, 85 P.

[1] Judovits, N., and Verzar, F., *Biochem. Z.*, 1937, **292**, 182.

[2] Verzar, F., and Laszt, L., *Biochem. Z.*, 1935, **276**, 11.

[3] Verzar, F., and McDougall, E. J., *Absorption from the Intestine*, Longmans, Green and Co., 1936.

[4] Barnes, R. H., Wick, A. N., Miller, E. S., and MacKay, E. M., PROC. SOC. EXP. BIOL. AND MED., 1939, **40**, 651.

TABLE I.

Exp.	Group	Sex	No. Rats in Group	Days	Avg body wt		Food intake rat/day, g	Fat intake rat/day, g	Stool fat rat/day, g	Stool fat % of food fat	Stool rat/day, g	Urine excretion per day 2-day period, g
					at start, g	at end, g						
1.	Controls	♀	9	14	128	142	8.6	1.03	.089	8.7		1.3
	Adrenalectomized	♀	7	14	126	132	7.0	0.83	.092	11.0		9.1
	Controls	♂	10	14	166	216	12.9	1.54	.159	10.3		2.8
	Adrenalectomized	♂	7	14	166	177	10.0	1.19	.135	11.3		21.0
2.	Controls	♀	5	14	142	161	6.8	1.63	.017	10.4	.26	
	Adrenalectomized	♀	5	14	145	150	7.4	1.78	.017	9.5	.28	
	Controls	♂	10	14	168	186	8.8	2.11	.113	5.4	.83	
	Adrenalectomized	♂	8	14	170	165	6.1	1.47	.053	3.6	.82	

fat ingested in the diet. This, of course, involves an error due to fat secretion by the lower intestinal tract, but in this case, the amount of stool fat from this source is a negligible item.

In experiment 1, our stock diet[5] in the form of a dry mash and in experiment 2, a synthetic paste diet (5, page 183) was fed. As a drinking fluid, a 0.5% NaCl and 0.2% $NaHCO_3$ mixture was allowed *ad lib.* Eight out of 35 adrenalectomized rats did not survive this regime. Data on these are not included in the averages. Evidence of adrenal insufficiency is found in the low weight gain of the adrenalectomized groups and the marked diuresis in these animals (Table I, Exp. 1).

The adrenalectomies and sham operations on the control rats were performed as usual.[5, 6] Food intake and stool collection observations were commenced a day later. The stools were collected over 24-hour periods and dried for analysis. "Fat" refers to the petroleum ether extract dried at 100°C, of either the food or the stools, as the case may be.

The data presented in Table I show very clearly that, under the conditions of these experiments, the absorption of fat is not influenced by adrenalectomy. Nor, if we assume that all of the stool fat is secreted by the intestinal mucosa, is there evidence that this excretion of fat is affected by removal of the adrenal glands.

Summary. Under fairly normal conditions of nutrition, salt solution being supplied as drinking fluid to maintain the adrenalectomized rats, adrenalectomy has no influence on the absorption of fat from the food. With adequate food intakes, the removal of the adrenal glands does not affect the excretion of fat in the stool.

10900

Effect of Heterologous Antigonadotropic Sera on the Course of Pregnancy in Rats.

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Recently Thompson[1] has reported on abortion produced in dogs with serum of a dog immunized over a period of 3½ years with sheep pituitaries. Thompson's results which were obtained by intravenous injection were almost 100% positive. It is beyond doubt that certain antigonadotropic sera from pituitaries of animals may exert a strong antigonadotropic effect in a heterologous species as has been shown by the extensive investigations of Rowlands.[2] On the other hand, it has been established by Zondek and Sulman[3] that the antigonadotropic sera produced by immunization with prolan of human origin and prosylan of pregnant mares' blood have a strong species specificity, so that not even 0.5% of the homologous activity could be exerted against prolan of heterogeneous origin. The positive results of Thompson have prompted us to publish a short review of the negative results which were obtained in 1938.

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[5] MacKay, L. L., and MacKay, E. M., *Am. J. Physiol.*, 1927, **83**, 179.

[6] MacKay, E. M., and MacKay, L. L., *J. Exp. Med.*, 1926, **43**, 395.

[1] Thompson, K. W., *Endocrinology*, 1939, **24**, 613.

The experiments of Pencharz and Long,[4] Selye, Collip and Thompson,[5] Bergman,[6] *et al.*, have shown that hypophysectomy in rats more than 4 days after mating prevents nidation. Hypophysectomy between the 7th and the 10th day of pregnancy results in the death and absorption of the foetus. Later on, between the 10th and 20th day, hypophysectomy results either in death and absorption of the foetus or in delay of term from 21-22 to 24-26 days.

In comparison with the aforementioned results of hypophysectomy we tried to produce similar effects by the administration of antigonadotropic sera at various stages.

The antigonadotropic sera used were derived from goats immunized during 9 months with daily injections of 1000 RU of pregnancy urine prolan + 25 RU prolan of human retroplacental blood (contained in 50 mg acetone-dry-powder of the serum) + 10 RU of prosylan of human anterior pituitary prepared according to Wallen-Lawrence and v. Dyke.[7] The antigonadotropic sera were stored as an acetone-dry-powder,[8] 60 mg corresponding to 1 cc of serum. Its antigonadotropic activity amounted to 300 PAU,* against pregnancy urine prolan and 40 PSAU,† against human hypophyseal prosylan in 1 cc (60 mg).

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[2] Rowlands, I. W., *Proc. Roy. Soc.*, L. Ser. B, 1937, **121**, 517.

[3] Zondek, B., and Sulman, F., PROC. SOC. EXP. BIOL. AND MED., 1937, **36**, 712.

[4] Pencharz, R. I., and Long, J. A., *Science*, 1931, **74**, 206; *Am. J. Anat.*, 1933, **53**, 117.

[5] Selye, H., Collip, J. B., and Thompson, D. L., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 689; **31**, 82.

[6] Bergman, F., *Act. Brev. Neerl.*, 1934, **4**, 81.

[7] Wallen-Lawrence, Z., and v. Dyke, H. B., PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 956; *J. Pharm. and Exp. Therap.*, 1931, **43**, 93.

[8] Zondek, B., and Sulman, F., PROC. SOC. EXP. BIOL. AND MED., 1937, **36**, 708.

* 1 PAU = 1 prolan anti-unit is the minimum amount of anti-prolan which annihilates the gonadotropic effect of 1 RU of prolan (estrus reaction) (*cf.* 8).

† 1 PSAU similarly defined (*cf.* 8).

The female rats were kept in separate cages and vaginal smears were taken daily. A regular cycle established during one month, the animals were mated and the development of impregnation and pregnancy determined according to Evans' technic for vitamin E test: (a) Impregnation was verified by the presence of spermatozoids in the smear and the formation of the typical vaginal plug. (b) Nidation was verified by the presence of red blood corpuscles in the smear 10-13 days later. (c) The progress of pregnancy was verified by the increase of the weight curve recorded daily.

First Series. (Table I.) The pregnant rats received 1 cc of multivalent antigonadotropic sera daily, starting on the 10th-13th day of pregnancy. The total amount of antigonadotropic sera given varied between 3000 PAU + 400 PSAU and 4500 PAU + 600 PSAU. In no case could we observe any symptoms due to the exclusion or inhibition of the pituitary system, as brought about by hypophysectomy; delivery was at term, the animals born were normal and developed normally. Since the objection might be raised that the injections were started too late to prevent the pregnancy from running its normal course we performed another series of experiments beginning the injections earlier and using double doses of antiprolan.

TABLE I.

First Group of Rats Receiving 300 PAU+40 PSAU Daily During Pregnancy.

No. of rat	Date of impregnation	First injection	Term of delivery	Duration of pregnancy days	Total of antigonadotropic sera given
R 1	10-1-38	20-1-38	1-2-38	22	3,600 PAU + 480 PSAU
R 2	11-1	23-1	2-2	23	3,300 ” + 440 ”
R 3	10-1	22-1	1-2	22	3,000 ” + 400 ”
R 4	13-1	23-1	3-2	22	3,600 ” + 480 ”
R 5	8-1	18-1	30-1	22	3,600 ” + 480 ”
R 6	8-1	18-1	30-1	22	3,600 ” + 480 ”
R 7	21-1	23-1	died		
R 8	15-1	23-1	6-2	23	4,500 ” + 600 ”

Second Series. (Table II.) Female rats with normal cycles were given daily injections of 2 cc of antigonadotropic sera corresponding to 600 PAU + 80 PSAU. This considerable amount of antigonadotropic factor was unable to interfere with the normal course of the cycle which regularly returned after periods of 4-6 days. A period of antigonadotropic treatment of 6 to 38 days having elapsed, the rats were mated and the injections continued. In spite of the considerable amounts of antigonadotropic serum injected, varying between 16,200 PAU + 2,160 PSAU and 36,800 PAU + 4,800 PSAU in all, the course of pregnancy was normal, no abortion occurred, the litters were born and developed normally.

TABLE II.
Second Group of Rats Receiving 600 PAU + 80 PSAU Daily Before and During Pregnancy.

No. of rat	Date of impregnation	First injection	Term of delivery	Period of treatment preceding impregnation (days)	Duration of pregnancy (days)	Total of antigonadotropic sera given
R 1	6-3-38	28-2-38	27-3-38	6	21	16,200 PAU + 2,160 PSAU
R 2	14-2	8-2	7-3	6	21	16,200 ,, + 2,160 ,,
R 3	6-3	8-2	28-3	26	22	28,800 ,, + 3,840 ,,
R 4	18-3	8-2	9-4	38	22	36,800 ,, + 4,800 ,,
R 5	9-3	15-2	29-3	22	20	26,400 ,, + 3,520 ,,
R 6	24-3	18-2	died	36	—	—
R 7	sterile	15-2	—	—	—	—
R 8	6-3	8-2	28-3	26	22	28,800 ,, + 3,840 ,,

These experiments are a striking proof of the essential part which species specificity plays in the treatment with antigonadotropic sera. Relatively astonishing amounts of antigonadotropic rat units were unable to interfere with the normal course of cycle and pregnancy in rats, because heterologous sera were used which were directed against human prolan and prosylan. There is no doubt that positive results would have been obtained if homologous sera against prosylan of rat pituitaries had been used. It may be possible that antigonadotropic factor directed against the pituitaries of sheep, ox, etc., would have acted in the rats. Antifactor against human gonadotropic hormone, however, has an extremely high species specificity and does not exert any effect except as against homologous human prolan. According to our experience and compared with the results of Rowlands[2] the species specificity of the antigonadotropic sera is not governed by the same rules prevailing in serology, and mammalian relationship reactions established there do not apply to the antigonadotropic reaction. With regard to Thompson's[1] results we have to stress the fact that he injected his dogs with antigonadotropic sera intravenously, whereas we did ours subcutaneously.

Summary. Large amounts of antigonadotropic sera against human prolan and prosylan were daily injected into normal rats. This treatment neither interfered with the normal cycle nor with impregnation, nidation, delivery and normal breeding of the animals. These negative results are to be explained by the high species specificity of antigonadotropic sera directed against prolan and prosylan of human origin.

10901

Does the Antigonadotropic Factor Occur in the Organism under Physiological and Pathological Conditions?

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The question as to whether the antigonadotropic factor is present in the organism of humans as well as animals under physiological conditions is one of fundamental importance. On this depends the question whether the formation of the so-called anti-hormones should be considered a regulatory mechanism (in accordance with Collip's definition) working in the organism under normal conditions, or whether it is a protective reaction somewhat like the immunity reaction.

We attempted to prove the presence of antigonadotropic substances in organisms which had not been subjected to preliminary treatment with gonadotropic hormone. We had the choice of two technics: (1) The serological method *(in vitro)*, and (2) the gonadotropic anterior pituitary reactions[1] *(in vivo)*. The negative results yielded by the use of the serological method have already been reported.[2]

In the following paragraphs we are going to describe our animal experiments.

1. *Does the antigonadotropic factor occur in the blood of patients who suffer from ovarian insufficiency?* Collip[3] reported the occurrence of antigonadotropic substances in the blood of women who had not been subjected to preliminary treatment. Laroche and Simmonet[4] found the antigonadotropic factor (antiprolan) in the blood of one castrata and one oligomenorrhoic woman. We tried to corroborate these findings, examining, moreover, women who suffered from primary amenorrhoea, secondary amenorrhoea or sterility. Our results were, however, negative. We analyzed quantities of 1-9 cc of serum against 1-5 RU of prolan. In the above mentioned cases not even 1 PAU* could be detected in 9 cc of serum.

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[1] Zondek, B., and Aschheim, S., *Klin. Wsch.*, 1927, No. 6.

[2] Sulman, F., *J. Exp. Med.*, 1937, **65**, 1.

[3] Collip, J. B., *J. Mt. Sinai Hosp.*, 1934, **1**, 28; *Ann. Int. Med.*, 1935, **9**, 50.

[4] Laroche and Simmonet, *Compt. rend. Soc. Biol.*, 1936, **121**, 416.

* 1 PAU = 1 prolan anti-unit is the smallest amount of the antigonadotropic factor required to annihilate the gonadotropic effect (estrous reaction) of 1 RU prolan in the immature female rat (*cf.* Zondek, B., and Sulman, F., PROC. SOC. EXP. BIOL. AND MED., 1937, **36**, 708).

2. *Does the antigonadotropic factor occur in the blood of patients suffering from tumors?* Flaks and Ber[5] furnished data on the occurrence of antigonadotropic substances in the urine of patients suffering from tumors. We investigated the occurrence of antigonadotropic substances in the serum of carcinomatous and sarcomatous patients. The sera assayed contained nothing of the antigonadotropic substance (less than 1 PAU per 3 cc of serum).

3. *Does the antigonadotropic factor occur during pregnancy?* It is a well established fact that the nidation of the ovum, in primates, is accompanied by a sudden rise of the proportion of prolan in the organism.[6] The prolan concentration then gradually diminishes until the end of pregnancy. Nature thus provides us with an example of a physiological flooding of the organism with large amounts of prolan, and the continuous influx of this substance should provide an ideal condition for the formation of antiprolan. The progressive regular decrease of the amount of prolan with advancing pregnancy should be explained (as according to Collip's antihormone-buffer-theory) by the formation of antiprolan.

It is not without technical difficulty that the presence of antiprolan is demonstrated, especially as the large quantities of prolan which are still present in the blood obscure the activity of antiprolan. The estrone content of the pregnancy serum also interferes with the verification of the presence of antiprolan, if the procedure is carried out with the estrus method (prolan A). A special experimental set-up is, therefore, required. We may use one of the following methods:

(A) The human pregnancy blood may be examined during puerperium after prolan as well as estrone have disappeared from the blood. Experiments set up in this way showed that there was no antiprolan in the blood of the puerpera one week after delivery. Fluhmann[7] has reported similar results.

(B) The pregnancy blood may be examined in such animals (or the foeti of these animals) in whom, normally, the production of prolan during pregnancy does not reach measurable proportions, which may be due to the production of antiprolan. Thus the experiments were carried out with rats, but neither in the mother nor in the foetus could antiprolan be detected at the time of delivery.

(C) As the most useful method we had to consider the analysis of pregnancy blood according to the technic indicated by us[8] (dissociation of the prolan-antiprolan mixture). If a prolan-antiprolan mixture is subjected to ultraviolet irradiation or to the action of H_2O_2 or HCl, prolan is destroyed[9] while antiprolan is not affected[10] and can be proved by means of the usual method (inactivation of freshly added prolan[8]). Ultraviolet irradiation and H_2O_2 did not, however, completely destroy the prolan so that this method was not practicable either. Neither was the experiment with HCl fully convincing as to its results, since the concentration of HCl necessary to destroy prolan was such as could equally destroy antiprolan. If a medium is used which contains little protein and electrolyte buffer it is, indeed, possible to destroy the urine-prolan with n/10 to n/15 HCl (during 20 hours at room temperature) without the antiprolan (from serum) which has been added being affected. In the serum prolan, a medium which contains much protein, quantitative destruction of prolan B by means of n/10 to n/15 HCl did not, however, succeed; n/5 HCl had to be used ($pH = 1.1$ at 37°C for 20 hours) in order completely to destroy the prolan in the serum. But in this way antiprolan was also destroyed. We, therefore, abandoned this method and chose the more successful procedure of dissociating pregnancy serum with NaOH.[8] We did not use non-treated pregnancy serum but prepared an acetone-dry-powder (according to the technic described[2]) from the fluids we wanted to assay: pregnancy blood, pregnancy urine, retroplacental blood, placental blood, amniotic fluid, puerperium blood. These types of dry-powder contain no estrone, while the concentration of prolan and antiprolan is practically the same as that of the serum (60-80 mg are equivalent to 1 cc of serum). If kept in the exsiccator these powders maintain their titers with reference to prolan or antiprolan for years, which greatly facilitates repeated tests and titrations even for the minutest amounts of prolan or antiprolan. Addition of NaOH to this powder very easily destroys antiprolan, while prolan is not affected, provided that the adequate concentration is adhered to.

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[5] Flaks, J., and Ber, A., *Dwutygodnika Medycyna* (Poland), No. 2, 1939.

[6] Aschheim, S., and Zondek, B., *Klin. Wschr.*, 1928, No. 30, 31.

[7] Fluhmann, C. F., *Tr. Am. Gyn. Soc.*, 1935, **60**, 237.

[8] Zondek, B., and Sulman, F., PROC. SOC. EXP. BIOL. AND MED., 1937, **37**, 343.

We started from the hypothesis that our acetone-dry-powders prepared from the various fluids of the pregnant human organism must necessarily contain antiprolan masked by prolan. We, therefore, arranged 15 experimental series in which NaOH in varying concentrations (from n/5 to n/50) was added to the acetone-dry-powders. Any amount of antiprolan present in the powder would have necessarily been destroyed by NaOH, thus increasing the gonadotropic titers of the dry-powders. The experimental set-up was the following:

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[9] v. Euler, H., and Zondek, B., *Scandin. Arch. Physiol.*, 1934, **68**, 232; Zondek, B., *Hormone d. Ovariums u. d. Hypophysenvorderlappens*, J. Springer, Vienna, 2nd ed., 1935, p. 242.

[10] Zondek, B., and Sulman, F., PROC. SOC. EXP. BIOL. AND MED., 1937, **37**, 193.

First experimental series: titration of prolan. Increasing doses of acetone-dry-powder from pregnancy blood, etc., were dissolved in n/10 NaCl and kept at room temperature for 24 hours, after 2 drops of brom-thymol blue had been added in order to check up the pH concentration. Then the neutral solutions were injected into infantile female rats, in 6 doses over a period of 36 hours, and in this way the prolan A titer was determined (estrus reaction).

Second experimental series: proof of antiprolan. Corresponding amounts of serum-dry-powder were dissolved in NaOH of varying concentrations (n/5 to n/50) in order to destroy any antiprolan which might happen to be present. They were also kept at room temperature for 24 hours after addition of brom-thymol blue. After this, the alkaline solution was neutralized by adding some drops of normal HCl and the gonadotropic titer determined according to the method mentioned above. As long as n/50 to n/15 NaOH was used no deviation of the gonadotropic titer was observed. The gonadotropic titer decreased, however, as soon as n/10 to n/5 NaOH was used, since the increasing NaOH concentration destroyed the prolan. With no NaOH dilution we did, however, achieve an increased prolan titer of our dry-powders, as should have been expected if antiprolan were present.

Summary. We may conclude from the results of the above experiments that no antiprolan is produced in the human organism during pregnancy. This has been ascertained in connection with the blood during the first as well as the last months of pregnancy, during delivery and one week postpartum. It is, therefore, evident that the organism does not use the antigonadotropic factor as a regulatory mechanism under physiological conditions. Antiprolan is exclusively elaborated in the organism after a type of prolan foreign to the species has been administered. Antiprolan formation must, therefore, be considered as a protective mechanism on pathological lines, as is similarly found in the immunity reactions, but not as a regulatory measure with reference to endogenous hormonal conditions. In accordance with this, no antiprolan has been proved to be present either in individual organs or in the blood of normal, amenorrhoic or sterile women or patients suffering from tumors.

10902

Production of Immunity to Poliomyelitis Virus in Motor Cells of the Monkey's Spinal Cord.*

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The following experiments constitute a demonstration that, as in the case of the olfactory bulbs already reported,[1] certain motor cells in the spinal cord of the rhesus monkey may be rendered resistant to the effects of invasion by poliomyelitis virus by means of procedures which produce alterations in their normal metabolism. Eighteen animals were subjected to section of the sciatic nerve in the sciatic notch at intervals varying between 3 and 57 days previous to the induction of complete leg paralysis by intranasal inoculation of poliomyelitis virus. A series of 6 uninoculated controls were operated upon in a similar fashion and sacrificed at intervals between 6 and 30 days.

These animals may be grouped under two headings. The first includes those monkeys in which the nerve section either produced no effect upon the susceptibility of the cells involved, or may have slightly enhanced it. This situation was found in 3 cases where nerve section had preceded leg paralysis by 3-4 days. In this group the cells of the operated and control sides were completely destroyed and it appeared that the destruction of the former had preceded that of the control cells.

Exactly the opposite effects were observed in the second group of 15 animals in which nerve section had been carried out 6-57 days previous to leg paralysis. In these cases the entire anterior horn of the control side was destroyed while on the operated side the vast majority of the cells giving rise to the sciatic nerve were spared. In the cases of longest duration of nerve section (13-57 days) the differences between the two sides were most striking (Figs. 1 and 2). As might be expected from the analysis of the cases in the first group, showing the opposite effect, the sparing was less extensive in those animals where nerve section was of shorter duration (6-8 days). Here sparing was confined to those levels in which the majority of the cells were concerned with the formation of the sciatic nerve.

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* Supported by a grant from the Commonwealth Fund.

[1] Howe, Howard A., and Bodian, David, *Proc. Internat. Congress for Microbiology*, New York, 1939.

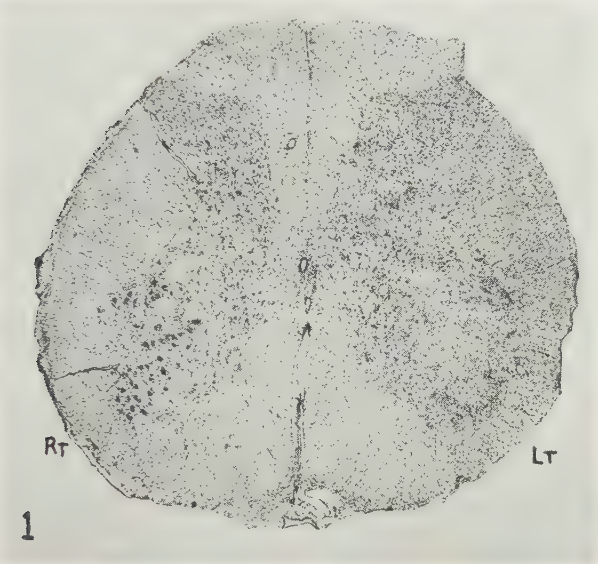
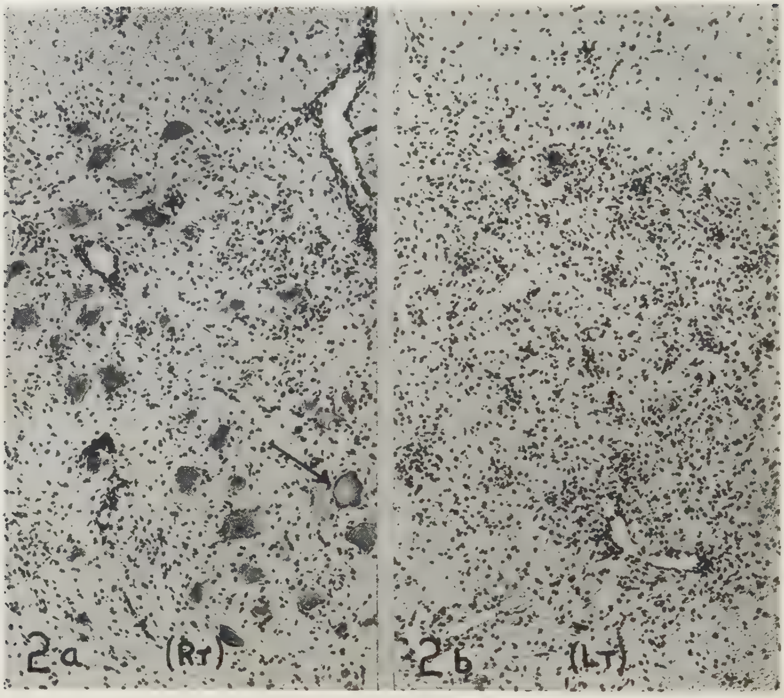


FIG. 1.

Lumbar cord of an animal in which the right sciatic nerve was sectioned 25 days prior to leg paralysis. Almost complete sparing of cells on the operated side.

The period of refractivity in the motor cells corresponds quite closely to the appearance of a morphological change known as "chromatolysis" or the "axone reaction". This reaction, which ensues upon section of the axone, is demonstrable principally as a reduction in the amount of Nissl substance in the cell cytoplasm. The exact nature of the physiological change which is mirrored in chromatolysis is obscure, but there are numerous reasons for believing that it involves an increase in the metabolic activity of the nerve cell itself. The reaction is apparent a few days after nerve section, but reaches a maximum in 10-20 days depending upon the amount of trauma inflicted upon the nerve and the distance from the cell body at which section takes place. For example, in 3 animals section of the upper lumbar roots at their emergence from the intervertebral foramina only 4 days previous to complete leg paralysis resulted in the production of a refractory state in the majority of the nerve cells chromatolyzed. This is several days earlier than the time at which a similar result could have been obtained by section of the sciatic nerve as far distal as the sciatic notch. This suggests that the state of refractivity to virus is indeed the result of the axone reaction, since its appearance varies in time with the latter. After the passage of several months the nerve cell may eventually regain its



FIGS. 2a AND 2b.

Figs. 2a and 2b are the right and left anterior horns of the lumbar cord of another monkey in which the right sciatic nerve was sectioned 18 days prior to complete leg paralysis. Fig. 2b shows the normal control side on which every cell appearing in the section was destroyed. Fig. 2a is taken from the same section and shows the operated side. Although practically every cell is spared, the presence of typical perivascular and interstitial lesions indicates the presence of virus on this side. Most of the cells are showing beginning recovery from chromatolysis but the cell indicated by the arrow is at the height of the reaction.

normal appearance. The correlation of the virus-refractory state with these late stages is yet to be made.

At this time one can do little more than indicate the significance of the above findings without attempting a discussion of their nature. There has been induced a reversible change in the metabolism of the nerve cell which renders it relatively refractory to the destructive effect of virus invasion. Nevertheless, such refractivity is probably only relative. This is suggested by the observation that compact chromatolytic groups are more resistant than scattered individual chromatolytic cells. In such compact groups even non-chromatolytic cells are sometimes spared. Two tentative conclusions appear to follow from the above findings: (1) that the increase in concentration of the virus occurs within the nerve cell itself and is possibly dependent upon some substrate which is reduced in chromatolytic cells, (2) that there is a threshold concentration, not achieved in the central portion of compact chromatolytic cell groups, which must be reached before the virus can become destructive. Heretofore an immunity of the cells of the CNS has been achieved only in the presence of active poliomyelitis virus. The real importance of these experiments lies in their demonstration of the fact that resistance to destruction by virus may be evoked in the normal cell without antecedent exposure to the infective agent.

10903

Surgical Production of Collateral Intracranial Circulation. An Experimental Study.

WILLIAM J. GERMAN AND MAX TAFFEL. (Introduced by S. C. Harvey.)

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Beck[1, 2, 3] and O'Shaughnessy[4, 5, 6] have demonstrated that an effective collateral blood supply to the heart can be established by the use of pedicled muscle and omental grafts. Others[7, 8, 9] have similarly employed the omentum to augment the circulation to the kidneys. The present experiments[10] were performed to determine whether an accessory arterial blood supply from an extracranial source could be provided for the brain by applying to its cortical surface a pedicled graft of temporal or suboccipital muscles.

Monkeys *(Macaca mulatta)* weighing 2 to 3 kilos were used as experimental animals. The operative approach and technic, as well as the sequence of the procedures, varied slightly during the course of

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[1] Beck, C. S., *Ann. Surg.*, 1935, **102**, 81.

[2] Beck, C. S., and Tichy, V. L., *Am. Heart J.*, 1935, **10**, 849.

[3] Mautz, F. R., and Beck, C. S., *J. Thoracic Surg.*, 1937, **7**, 113.

[4] O'Shaughnessy, L., *Brit. J. Surg.*, 1936, **23**, 665.

[5] O'Shaughnessy, L., *Lancet*, 1937, **1**, 185.

[6] O'Shaughnessy, L., Slome, D., and Watson, F., *Lancet*, 1939, **1**, 617.

[7] Davis, H. A., and Tullis, I. F., Jr., PROC. SOC. EXP. BIOL. AND MED., 1939, **40**, 161.

[8] MacNider, W. deB., and Donnelly, G. L., PROC. SOC. EXP. BIOL. AND MED., 1939, **40**, 271.

[9] Mansfield, J. S., Weeks, D. M., Steiner, A., and Victor, J., PROC. SOC. EXP. BIOL. AND MED., 1939, **40**, 709.

[10] German, W. J., and Taffel, M., *Proc. Third Internat. Neurol. Congress*, Copenhagen, Denmark, 1939, in press.

the experiments, but the main objectives in each instance remained the same. After either preliminary or concomitant ligation of one or more of the main arteries to the brain, the superior border of the temporal muscle was separated from its origin and reflected to expose most of the underlying squamous portion of the temporal bone. The muscle was left attached at its insertion and its blood and nerve supply not disturbed. A large defect was made in the temporal bone, the exposed dura excised, and the underlying arachnoid peeled gently away from the cerebral convolutions. The periosteal surface of the temporal muscle was scarified and applied directly to the denuded brain. The superior border of the muscle was sutured to the remaining free edge of the dura at the margin of the bony defect. No sutures were placed between the muscle and the brain itself. In 2 of the animals pedicled grafts of the suboccipital muscles were placed over the cerebellar hemispheres in addition to the bilateral temporal grafts. At varying intervals the animals died or were sacrificed and the accessory circulation was studied by arterial injection methods, by specimens cleared according to the Spalteholz technic, and by histological sections.

I. The first group of experiments was designed to test the functional efficiency of the artificially produced collateral blood supply. The serial ligations of the 4 major arteries were all performed in the neck.

a. Monkey No. 8.

Oct. 11, 1938	Ligation of left internal carotid artery.
" 12	Left temporal muscle graft applied.
Nov. 8	Ligation of right vertebral artery.
" 9	Right temporal muscle graft applied.
Dec. 6	Partial ligation of right internal carotid artery. Some difficulty with breathing noted during operation, but animal made complete recovery.
" 20	Complete ligation of right internal carotid artery. Respirations irregular and labored. Death.

At autopsy both vertebral arteries were ligated in the neck and India ink injected into each common carotid artery. All the larger arteries of the brain were later found to contain the ink. The muscle grafts (6 and 9 weeks old) were firmly adherent to the surface of the brain. Histological studies revealed these grafts to be joined to the brain by a loose vascular fibroblastic layer which was infiltrated throughout with a moderate number of mononuclear cells and occasional polymorphonuclear leukocytes. The cellular infiltration extended deeply into the muscle graft but was inconspicuous in the underlying cerebral cortex. In serial sections, large and small thin-walled blood vessels were seen to course from the muscle through the inflammatory connecting zone where they communicated with surface pial vessels, or directly with the cortical vessels emerging from the substance of the brain. India ink particles were visualized in most of the arteries of the brain, the muscle, and the connecting zone.

b. Monkey No. 72.

Dec. 7, 1938	Ligation of left internal carotid artery; left temporal muscle graft.
Jan. 4, 1939	Ligation of right vertebral artery; right temporal muscle graft.
Feb. 1	Partial ligation of right internal carotid artery.
” 15	Complete ligation of right internal carotid artery.
Mar. 1	Partial ligation of left vertebral artery.
“ 16	Complete ligation of left vertebral artery.
Apr. 4	Animal sacrificed. The monkey suffered no obvious ill effects from any of the operative procedures.

Autopsy: The calvarium was rongeured away and silver clips applied intracranially to both vertebral and both internal carotid arteries. A 1% aqueous solution of trypan blue was then injected slowly, first into one common carotid artery and then the other. The dye was seen to course slowly through the temporal muscle, cross over directly from the muscle into the large pial vessels and then spread in a fan-like fashion over the surface of the brain. After the injection of about 15 cc, some of the dye passed upwards through the vessels on the medial wall of the cerebral hemisphere to meet the circulation of the dye coming directly over the convex surface of the brain. This seemed to indicate the passage of the dye from the muscle graft through intermediary vessels to the circle of Willis, and thence to the anterior cerebral arteries. All the above phenomena were observed on both sides.

When the brain was removed the arteries and arterioles everywhere were stained with the dye. The muscle grafts were solidly fused and well stained. The vascular connections between the muscle and the brain were demonstrated in thin sections cleared by the Spalteholz method.

On microscopic examination the connecting zone on the right side (12-week-old graft) was composed of a layer of fibroblasts rich in blood vessels. The cellular infiltration was present but slight. Between the connecting zone and the muscle there was a layer of dense pink staining fibrous connective tissue which communicated frequently with the fibrous sheaths between the muscle fasciculi. This layer, with wavy and irregular surfaces, appeared to be the regenerated deep temporal fascia and periosteum which had been scarified prior to the application of the graft. The histological picture on the left side (16-week-old graft) was essentially the same except that the fibroblasts in the intermediate zone were more mature, more closely packed, and began to resemble adult connective tissue cells.

c. Monkey No. 9. Control.*

Oct. 19, 1938	Ligation of left internal carotid artery.

Nov. 16	Ligation of right vertebral artery.
Dec. 14	Partial ligation of right internal carotid artery.
” 28	Complete ligation of right internal carotid artery.
Jan. 11, 1939	Partial ligation of left vertebral artery.
” 25	Complete ligation of left vertebral artery.

The animal continued to thrive despite the fact that the 4 major vessels had been ligated. No muscular weakness or paralyses were noted.

March 22, 1939. Thorotrast was injected into common carotid arteries and an effort made to visualize the natural anastomotic channels radiographically. This was not successful. Animal sacrificed.

Definite collateral vascular channels joining the brain and the adherent temporal muscle have been demonstrated both in the histological sections and by the injection technic. Inasmuch as the ligation of both internal carotid and both vertebral arteries was well tolerated by the control animals, no definite conclusions can be drawn from these experiments concerning the efficiency of the accessory extracranial vascular communications.

II. In an effort to exclude as much as possible the naturally existing collateral arterial channels proximal to the circle of Willis, the arterial occlusions were performed intracranially. Two of the major vessels were clipped at operation, and the remaining 2 at autopsy immediately prior to the injection with the dye. In animals No. 73 and No. 90 the procedure was varied by preliminary ligation of these vessels in the neck and by the application of suboccipital muscle grafts to the cerebellum. In these latter 2 animals the internal carotid arteries were at no time obstructed intracranially.

a. Monkey No. 73.

Dec. 7, 1938	Ligation of left internal carotid artery.
Jan. 4, 1939	Ligation of right vertebral artery.
Feb. 1	Partial ligation of right internal carotid artery.
” 15	Complete ligation of right internal carotid artery.
Mar. 1	Partial ligation of left vertebral artery.
” 16	Complete ligation of left vertebral artery.
” 29	Left temporal muscle graft applied.
” 31	Right temporal muscle graft applied.
May 10	Both vertebral arteries occluded intracranially with silver clips; bilateral suboccipital muscle grafts applied to cerebellum. All of the above procedures were well tolerated.
June 23	Animal sacrificed. Common carotid arteries injected with India ink and gelatin mixture.

At autopsy the arterioles in the brain were well outlined with the India ink. The temporal and suboccipital grafts were firmly adherent to the cerebral and cerebellar hemispheres respectively.

Cleared thin sections demonstrated abundant vascular communicating channels between the brain and all the muscle grafts.

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* In addition, animals No. 73 and No. 90 served as controls for this experiment during the period from December to May (protocols below).

b. Monkey No. 90.

In this animal the serial operations as well as the time intervals between them were in all respects identical with those of monkey No. 73. Postmortem examination revealed the arterioles of the brain to be filled with the ink and gelatin mixture. The temporal and suboccipital muscle grafts were firmly adherent.

c. Monkey No. 57.

Apr. 27, 1939	Right internal carotid artery occluded intracranially with silver clip; right temporal muscle graft applied.
May 17	Left internal carotid artery occluded intracranially with silver clip; left temporal muscle graft applied. Following this operation the animal became unresponsive, and appeared as though decerebrate. There was a complete paralysis of the third cranial nerve on the left side. Death occurred on May 19, 1939.

At autopsy the calvarium was rongeured away, and silver clips applied intracranially to both vertebral arteries. A trypan blue solution was injected into each common carotid artery. On the right side (3-week-old graft) the dye was seen to pass readily from the temporal muscle to the brain exactly as described in animal No. 72. On the left side (2-day-old graft) none of the dye crossed from the left temporal muscle directly to the brain. Eventually the left half of the brain was stained with dye which had come from the right side. On cross section the dye was again concentrated chiefly in the right side. The right temporal graft was firmly adherent to the brain. The left was attached only loosely by delicate fibrinous adhesions.

Results. An accessory and abundant arterial blood supply for the brain was experimentally produced in monkeys by the use of pedicled temporal and suboccipital muscle grafts. The newly formed connecting channels between the muscles and the brain have been demonstrated to be patent as early as 3 weeks after the application of the graft. Inasmuch as the cervical ligation of both internal carotid and both vertebral arteries was well tolerated by the control animals, no definite conclusions can be drawn from these experiments concerning the functional efficiency of these new vascular communications.

The grafts were solidly attached to the underlying brain by a vascular layer of fibroblasts which gradually assumed the characteristics of adult connective tissue cells.

The clinical application of this method for supplementing intracranial circulation should be preceded by further experimental observations including long-term studies, which are now in progress. Cerebral arteriosclerosis, recurrent cerebral thromboses, and intracranial aneurisms requiring internal carotid ligation suggest themselves as possible examples where augmentation of the intracranial circulation would be desirable.

10904

Effects of Administering Mouse Anterior Pituitary to the Newt and the Frog.*

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Induction of extra-seasonal ovulation in anurans and urodeles has been secured by injecting extracts of mammalian anterior pituitary[1] or of human pregnancy urine[1] although in some instances no response occurred.[1, 2] Attempts to cause the reaction with implants of fresh mammalian gland (sheep, cattle, dog, guinea pig, rat, rabbit[3]) have given negative results. Further tests of fresh substance were, therefore, made in which mice were donors and 2 amphibians, *Triturus viridescens* and *Rana pipiens,* hosts.

Triturus viridescens as hosts. (Experiments performed by E. DeF.) Between October, 1938, and March, 1939, 24 adult female newts received implants of fresh anterior pituitaries of adult male mice in varying amounts and spaced at varying intervals. Five died on the 2nd, 5th or 6th day of the experiment and one at autopsy had an ovary filled with atretic eggs. Of the 18 remaining, 6 ovulated and 5 of these laid eggs (Table I). The first egg-laying occurred at an average of 11.2 days. The average number of eggs ovulated was 10.3 and laid was 4.6 per newt. The average number of APs given was 12.5, a dosage of 12.6 mg of substance.

As controls 16 newts received implants of newt whole pituitaries spaced to correspond with the mouse implants. Four did not ovulate, but 3 of these died within 2 to 5 days after treatment and in the 4th the ovaries were immature. The remaining 12 laid eggs after an average of 6.1 glands (approximately 0.84 mg of substance[4]) and of

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* Aided by a grant from the Rockefeller Foundation.

[1] See Adams, A. E., *Studies in Experimental Zoology,* 1936, Edwards Brothers, Ann Arbor, pp. 65-67, and Rugh, R., *J. Exp. Zool.,* 1935, **71**, 149, Table 3 for bibliography.

[2] Adams, A. E., Proc. Soc. Exp. Biol. and Med., 1931, **28**, 677; Baker, L. C., *J. Tenn. Acad. Sci.,* 1937, **12**, 206; Creaser, C. W., and Gorbman, A., *Copeia,* 1936, p. 91; Gallien, L., *Compt. rend. soc. biol.,* 1937, **124**, 874; Zondek, B., *Hormone des Ovariums und des Hypophysenvorderlappens,* J. Springer, 1935.

[3] Adams, A. E.;[2] Houssay, B. A., Giusti, L., et Lascano-Gonzalez, J. M., *Compt. rend. soc. biol.,* 1929, **102**, 864; Wills, I. A., Riley, G. M., and Stubbs, E. M., Proc. Soc. Exp. Biol. and Med., 1933, **30**, 784; Witschi, E., *Cold Spring Harbor Sympos. Quant. Biol.,* 1937, **5**, 180.

[4] Figure computed from data in paper by Adams, A. E., and Granger, B., Proc. Soc. Exp. Biol. and Med., 1938, **38**, 552.

TABLE I.

Triturus viridescens Given Intramuscular Implants of Adult Male Mouse Anterior Pituitaries or of Male Newt Whole Pituitaries.

No. of newts	Treatment	Anterior pituitaries		Day of autopsy	Eggs			Actual or estimated[1] wt of both ovaries (mg)
		No.	Total wt (mg)		laid	in body cavity or oviducts	in ovaries	
	Donor Mouse							
1	1 AP daily	11	12.78	12	13	0	109.0	474.00
1	1 ,, ,,	11	11.79	12	1	0	127.0	448.51
1[2]	1 ,, ,,	13	13.26	14	2	7	115.0	359.34
8[3]	1 ,, alternate days	15	avg 12.97	{31 34	0	0	avg 278.5	avg 693.65
1	2 ,, daily	16	11.60	9	1	1	176.0	575.72
1	2 ,, alternate days	12	14.14	12	6	22	93.0	453.80
2	2 ,, ,, ,,	16	avg 13.03	16	0	0	avg 170.5	avg 566.90
1[4]	Single dose	14	12.2	10	0	0	104.0	370.80
1[5]	,, ,,	15	12.8	10	0	0	135.0	461.80
1	,, ,,	24	11.8	13	0	9	151.0	462.52
	Donor Newt	Whole pituitaries						
1	1 AP daily	11	1.51[6]	13	77	0	35.0	456.96
1	1 ,, ,,	11	1.51	15	93	0	33.0	431.54
1[2]	1 ,, ,,	11	1.51	12	22	2	106.0	480.60
1[2]	1 ,, ,,	13	1.78	14	17	1	116.0	398.10
4	1 ,, alternate days	15	2.06	{31 34	avg 76.25	avg 1.5	avg 44.25	avg 371.93
1	2 ,, daily	12	1.64	7	37	0	44.0	300.38
1	2 ,, ,,	16	1.10	9	41	23	47.0	376.40
1	2 ,, alternate days	16	2.19	16	65	8	30.0	387.28
1	2 ,, ,, ,,	16	2.19	16	81	1	107.0	547.26

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[1] The *estimated* total weight of both ovaries was obtained by adding the actual weight of the ovarian remnants to the weight of the eggs that had been ovulated (number of eggs times the average weight of a single egg).

[2] Thyroidectomized 4 days before treatment.

[3] In one newt of this series, the 15 glands implanted consisted of 6 from female and 9 from male donors.

[4] Implants in body cavity.

[5] Subcutaneous implants in throat.

[6] Figure computed from data in paper by Adams and Granger, PROC. SOC. EXP. BIOL. AND MED., 1938, **38**, 552.

7.2 days after the first treatment. The average number of eggs ovulated was 64.9 and laid was 61.5 per newt.

Although only 6 of the newts receiving mouse pituitaries ovulated, in the 8 given 1 AP every other day for 30 days, the average weight of both ovaries (693.65 mg) was nearly twice as great as the average estimated total weight (371.93 mg) of the ovaries of the 4 control newts in this series (Table I). The increase occurred throughout the ovaries since the part containing young oögonia and yolkless oöcytes was 26.90% of the average total ovarian weight in the experimental newts and 25.13% in the controls. A single, large, yolk-laden egg from the experimental and control ovaries weighed the same, 2.42 and 2.41 mg, respectively, but the average number of such large eggs in the former (278.5) was more than twice that in the latter (122.0).

Rana pipiens as hosts. (Experiments performed by A.E.A. and B.G.) During December, 1938, and January, 1939, 9 adult female *Rana pipiens* were given fresh anterior pituitaries from adult male mice. The substance was injected in small amounts of distilled water into the body cavity or the dorsal lymph sacs of the host. Six controls received 3 or 4 fresh anterior lobes from *Rana pipiens* females and 4 received fresh mouse brain in amounts similar to those of mouse pituitary. A male injected with frog AP was placed with each female, except 2 given mouse brain, 1 or 2 days after injections began.

It is evident that ovulation did not occur in the 9 frogs given amounts of mouse AP ranging from 35.23 mg (43 APs) to 229.2 mg (269 APs) (Table II). All of them had mature ovaries at autopsy or laparotomy. The 3 which were laparotomized were given a single dose of 4 APs from female frogs (avg, 4.9 mg) 1 or 2 days after laparotomy and all ovulated 3 days later. The 6 controls receiving frog APs (avg dose, 5.37 mg) laid eggs or eggs were pressed from the cloaca on the first or second day after treatment. The 4 frogs given fresh mouse brain (41.31, 88.2, 96.39, 232.0 mg) did not ovulate or appear to suffer any toxic effects from the treatment.

From the experiments in which *Triturus viridescens* was the host, it appears that there is no "zoological specificity" of the gonadotropic hormones of the vertebrate anterior pituitary, for ovulation was secured, but in the series where *Rana pipiens* was the host and no ovulation occurred, the data favor the other concept. However, when previous experiments are considered where negative evidence has been superseded by positive, especially when the dosage has been increased, as in the reaction of toads to fish and frog anterior pitui-

TABLE II.
Rana pipiens Given Anterior Pituitaries from Adult Male Mice.

No. of frogs	Ant. pituitaries No.	Ant. pituitaries Wt (mg)	Treatment	Results
3	43	avg 35.23	5 doses in 6 days (6, 6, 12, 12, 7 APs)	Killed 8th day; no ovulation
11[1]	88	67.9	1 dose	Killed 9th day; no ovulation
1	90	76.02	4 doses in 5 days (36, 18, 18, 18 APs)	Escaped 14th day; no ovulation[2]
1	90	81.69	,, ,, ,,	Laparotomized 14th day; no ovulation[3]
1	100	88.8	1 dose	Laparotomized 7th day; no ovulation[3]
1	108	92.89	3 doses in 5 days (36 APs per dose)	Killed 13th day; no ovulation
1	269	229.2	3 doses in 6 days (100, 100, 69 APs)	Laparotomized 7th day; no ovulation[3]

[1] Splenectomized the day before treatment.

[2] This frog weighed 58.0 g at beginning of treatment. Attempts were made daily to press eggs from cloacal opening without effect. It is probable that its ovaries were mature.

[3] One or two days after laparotomy a single dose of 4 APs from adult female frogs (avg dose 4.9 mg) was administered and the frog ovulated 3 days later.

tary,[3] or of mice to frog anterior pituitary,[5] it seems wise to suspend judgment until further tests are carried out. Perhaps the dosage is not yet sufficient, for Gallien[2] estimated that extract from 1.6 to 3.0 g of fresh beef anterior lobe was necessary to induce ovulation in *Rana temporaria* comparable to that caused by 6 to 9 of its own glands, or only a few milligrams of substance. Moreover, these 2 hosts, *Triturus viridescens* and *Rana pipiens,* vary greatly in their reaction to gonadotropic hormones. The former ovulates readily after anterior pituitary from different species of newts, from *Rana pipiens,* from *Bufo terrestris,* from fowl, and after mammalian anterior pituitary extracts (phyone and hebin)[1] and pregnancy urine (Antuitrin-S),† whereas the latter responds without difficulty to homoplastic and heteroplastic treatment[6] but less easily or negatively to xenoplastic (newt) or dysplastic (fish, mammal) anterior pituitary substance.[7] Similar variations in the reaction to gonadotropic hormones occur in many of the vertebrates—other species and genera of anurans and urodeles, the 2 sexes of amphibians, birds and mammals, and also different mammalian forms (rats, mice, rabbits, guinea pigs).[8]

Even though a positive result was secured with mouse anterior pituitaries certain data indicate that the response occurs with some difficulty. For example, the percentage of newts ovulating is smaller, the number of eggs ovulated or laid per newt is much less, the ovulation occurs much later and the dosage required is greater than in controls given whole pituitaries from newts. It looks as if some barrier prevents as full a response after dysplastic treatment as that which occurs when a homoplastic one is given. Perhaps the release of the active principle of the pituitary substance is slowed due to the differences in the metabolism of warm- and cold-blooded forms or to antagonistic reactions between tissues of host and donor species.[9] However, the results secured in the series of newts given 1 mouse AP every other day for 30 days where marked increase in ovarian size occurred suggest that no immunity was set up in the hosts over this period. Those results and the cases where ovulation did occur (Table I) and those where the reproductive systems of infantile female mice responded to very large doses of *Rana pipiens* AP[5] support the idea that a certain threshold of hormones must be reached if the gonads are to react, especially if the donor is very far removed from the host in the evolutionary scale.

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[5] Adams, A. E., and Granger, B., PROC. SOC. EXP. BIOL. AND MED., 1938, **38**, 585; Adams, A. E., and Tukey, G., *Anat. Rec.*, 1938, **71**, 1 (see Table I); Zwarenstein, H., *Nature*, 1937, **140**, 588.

† We are indebted to Dr. Oliver P. Kamm of Parke, Davis and Co., for the Antuitrin-S.

[6] Rugh, R., *J. Exp. Zool.*, 1935, **71**, 149 (Tables I and II); *Physiol. Zool.*, 1937, **10**, 84; Creaser, C. W., and Sheolnek, M., *Mich. Acad. of Sci., Arts and Letters*, 1938, **24**, 85, Table II.

[7] Adams, A. E., and Granger, B.;[4] Creaser, C. W., and Gorbman, A.;[2] Creaser, C. W., and Sheolnek, M.;[6] Wills, I. A., Riley, G. M., and Stubbs, E. M.[3]

[8] Allen, E., Editor, *Sex and Internal Secretions*, 2nd Edit., 1939, Williams and Wilkins, Chap. IX, XVI.

[9] Loeb, L., *Physiol. Rev.*, 1930, **10**, 547.

Summary. *Triturus viridescens* ovulated extra-seasonally after implants of mouse anterior pituitary averaging 12.6 mg, although the reaction was slower and less complete than that after approximately 0.84 mg of newt whole pituitary substance, or the ovaries increased markedly in size if the above dosage of mouse pituitary was distributed over a long period (30 days). *Rana pipiens,* however, gave no ovulatory response although much larger doses (35.23 to 229.2 mg) of mouse anterior pituitary were given.